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PSEUDO-ALGAL NODULES IN THE GREENFIELD DOLOMITE (UPPER SILURIAN OF OHIO)

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The Greenfield dolomite is the basal member of the Bass Island formation of the Upper Silurian Monroe division. The type locality is in the vicinity of Greenfield, northeastern Highland County, Ohio. It has been described by Carman[1] as a "drab, fine-grained dolomite with carbonaceous partings and commonly in beds of 2–6 inches, although at places with thicker beds or massive ledges. This massive phase is rough textured and vesicular, with corals and stromatoporoids, . . ." The thickness is not accurately known, but it is presumed to be between 75 and 100 feet.

In the old, and now abandoned, Rucker quarry, just east of Greenfield and across the line in Ross county, 53 feet of the Greenfield are exposed[2] in what may be considered the type exposure. Here are exhibited many interesting sedimentary rock structures such as mud-cracks, ripple-mark, stylolites, "cup-and-cone" concretions, and near the top of the section is a conglomeratic layer from 5 to 30 inches thick composed in places of rounded or flat pebble-like dolomitic nodules loosely cemented by dolomitic limestone (text figures 1 and 2). These nodular bodies are the special concern of these remarks.

They were first mentioned in print in 1871 by Edward Orton[3], who speaks of "a layer of concretions, from one to three inches in diameter, near the upper part of the section [in the Rucker quarry]." In 1915, Bownocker[4] located the layer in the massive 5-foot course six feet below the top of the exposure, and noted that it contained "a profusion of well rounded limestone pebbles, usually about an inch in diameter," and that "these resulted from wave action when the locality was at or near a shore line." The latest notice of them was in 1917 by Napper[5] who remarked that when broken the nodules show a concentric structure. Beyond these scattering and sketchy allusions the writer has failed to find other mention of these peculiar structures. His attention to them was drawn by Dr. J. E. Carman, and he was struck by their outward resemblance to the well-known "water-biscuits" of Squaw Island at the northern end of Canandaigua Lake, central New York[6], and to similar nodules formed by algal agency in fresh and brackish

[1]Carman, J. E., 1927, "The Monroe Division of Rocks in Ohio," *Jour. Geol.*, Vol. 35, p. 486.

[2]Bownocker, J. A., 1915, "Building Stones of Ohio," *Geol. Surv. Ohio, 4th Ser., Bull.* 18, p. 43.

[3]Orton, E., 1871, "The Geology of Highland County," *Geol. Surv. Ohio, Progress Rep. for 1870*, p. 290.

[4]Bownocker, J. A., *op. cit.*, p. 44.

[5]Napper, C. W., 1917, "Concretionary Forms in the Greenfield Limestone," *Ohio Jour. Sci.*, Vol. 18, p. 11.

[6]Clarke, J. M., 1903, "The Water Biscuit of Squaw Island, Canandaigua Lake, N. Y., *54th Ann. Rep. N. Y. State Mus.*, 1902, Vol. 3, pp. 195–198, pls. 13–15.

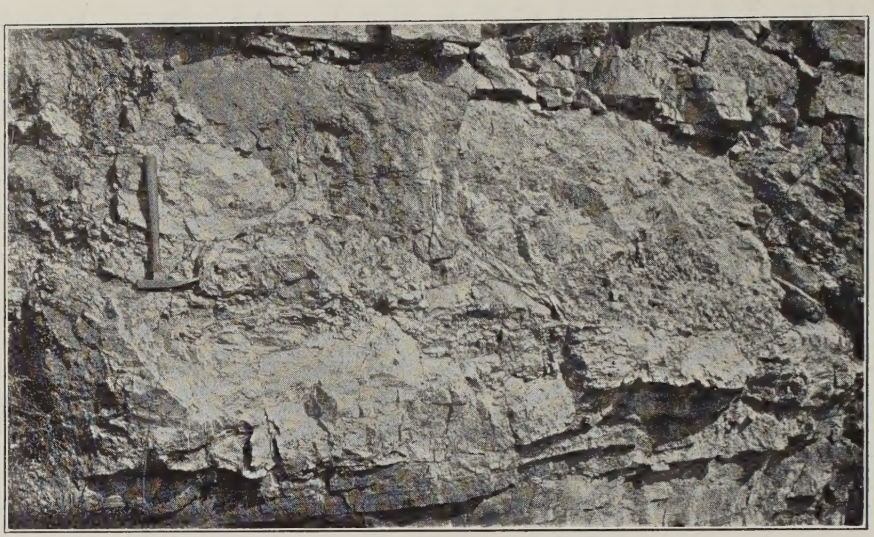

Fig. 1—A

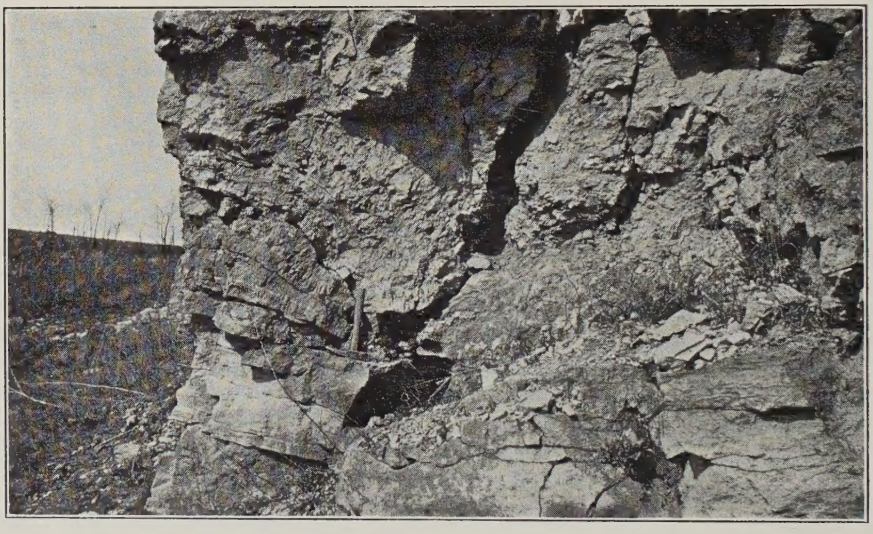

Fig. 1—B

Fig. 1. Zone of pseudo-algal nodules in the Greenfield dolomite, near top of east wall of old Rucker Quarry, east of Greenfield, Ohio: *a*, Base of hammer handle is just below a pocket of nodules; *b*, Nodular zone to left and right of hammer, with non-bedded dolomite with scattered patches of nodules, above and below, and with regularly-bedded dolomite at bottom and top. (Length of hammer, 15 inches.)

waters in many other localities, and which have been reported from many geologic horizons from Pre-Cambrian to Recent.

EXTERNAL FORM AND OCCURRENCE OF THE NODULES

They vary in shape and in size from nearly spherical balls 2 to 5 cm. in diameter (Plate I, Fig. 8) to flat, shingle-like pebbles from 2 to 6 cm. across and up to 1 cm. in thickness (Plate I, Fig. 9). The rounded ones frequently show one or more flat, subfacetted surfaces, as if they had been packed together in a plastic condition, and are simply agglomerated with more or less porous or fragmental dolomitic limestone between them. The flattened pebbles usually show a definite orientation, overlapping each other and often steeply inclined in the same manner as the flat pebbles on shingle beaches and shallow bottoms, athwart the general direction of wave motion and inclined in the direction whence it came, the whole mass constituting a sort of edge-wise conglomerate.

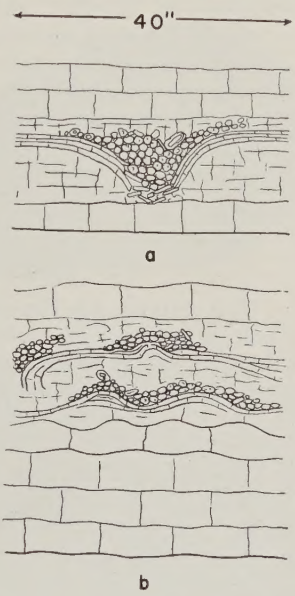

Fig. 2. Diagrammatic sketches of nodular zone in the Greenfield dolomite, old Rucker Quarry, east of Greenfield, Ohio: *a*, Area above hammer in Fig. *1a; b*, Area to right of hammer in Fig. *1b*.

STRUCTURE OF THE NODULES

The rounded nodules, as revealed by thin sections, are composed of concentrically arranged laminae of closely aggregated dolomite granules arranged about an irregularly shaped nucleus which is often a fragment of the same laminar material, or a very porous dolomitic mass of coarse granules (Plate I, figs. 1, 4). The lamination is strongly marked by narrow yellowish-brown limonitic bands which are darker than the prevailing drab tone of the nodules. In one ovoid specimen the nucleus was but half-filled with spongy dolomite, the rest being a plano-convex cavity with drusy calcite crystals. The limonitic bands appear in places as sharply defined continuous, or rarely discontinuous dark lines (Plate I, Fig. 2), in others as vague pale stainings of the dolomitic granules. An average nodule measuring 22 x 35 mm. in diameters has a porous nucleus 3 x 12 mm., with three strongly-marked dark laminae about 0.5 mm. think and from 2 to 5 mm. apart. Between each of the dark layers are from 10 to 15 thinner, finer ones, frequently forming distinct laminae separated from each other by open lenticular spaces partly filled with drusy calcite (Plate I, Fig. 4). The heavier laminae are usually circumferentially continuous, but the thinner intermediate ones may merge laterally with the ones above or below them, especially where they pass around the end of the longer axis. Many of the bands show a sharp peripheral boundary and grade centrally into the light zones.

The flat, shingle-like nodules show the same concentric banded structure, but with fewer open lenticular spaces and more even and regular lamination (Plate I, Fig. 10). The nucleus of each is a fragment of rock, a bit of broken-up stratum having the same laminated structure and averaging 7 mm. in thickness. The lower laminae of the nuclei are flat and undisturbed, but the upper ones (comprising about two-thirds of the thickness of the nucleus) form a zone of contorted layers with many cavities, as if the contortion occurred just before or simultaneously with the breaking up of the stratum, perhaps by subaqueous solifluction.

That the deposition of the concentric laminae around these fragments took place before the latter were re-arranged in their present situation, probably by wave-action, is shown by the separation of the coated fragments from each other by several layers of concentric laminae. The upper part of the layer of shingled pieces is covered by 15 to 50 mm. of laminae like those of the rounded and flat nodules and the nuclei of the latter, and which at first follow the irregular edges of the fragments closely but which slowly even out with the uppermost laminae only slightly undulant. This later deposit binds the shingled layer together fairly well and its general aspect is that of the physico-chemical encrustations on algal masses described by Bradley[7] in these terms:

> They resemble layers of algal deposit in thinning and in places wedging out on the steep or overhanging sides of dome-shaped protuberances, but unlike the algal deposits many continue as thin encrustations into the bottoms of pocket-like depressions or infolds and up the sides of the adjoining excrescences. Furthermore they differ from layers that are patently algal in tending toward a simpler surface form where many are superimposed. They fill the invaginations between algal colonies and bridge gaps between them, and above such bridges each succeeding layer becomes more uniform in thickness and so smooths out the surface contour.

COMPARISON WITH NODULES OF ALGAL ORIGIN

The criteria for the distinction of structures due to algal agency from those resulting from physico-chemical processes are not well-defined and can scarcely be said to have received much attention in print, especially for such cases as the present one where later diagenetic changes have occurred. However, comparison of the Greenfield nodules with authentic algal structures is of service in an attempt to discern their origin.

The shape of the more nearly spherical nodules and their gross concentric internal structure is strongly suggestive of algoid balls, but their microscopic character does not show features associated with known algal agencies, such as the minute spherical shells of the unicellular *Chlorellopsis*,[8] the fine, loose tubules of such

[7]Bradley, W. H., 1929, "Algal Reefs and Oolites of the Green River Formation," *U. S. Geol. Surv. Prof. Paper* 154, p. 209.

[8]Bradley, *op. cit.*, pl. 32.

EXPLANATION OF PLATE I

Algal and Pseudo-Algal Nodules

Fig. 1. Cross-section of rounded nodule from the Greenfield dolomite with porous nucleus, × 1.

Fig. 2. Part of same specimen showing uniformity of the laminae, × 7.

Fig. 3. Part of same specimen showing lenticular drusy cavities between laminae, × 7.

Fig. 4. Cross-section of rounded nodule from the Greenfield dolomite, with fragment of laminated dolomite as nucleus, × 1.

Figs. 5 and 6. Cross-section of *Zonotrichites* nodules from Upper Cretaceous fresh-water beds, Willow Creek, east of Redmond, Sevier County, central Utah, collected by E. M. Spieker, × 1. The nucleus of Fig. 5 is a fragment of pink rhyolite.

Fig. 7. Cross-section of *Zonotrochites* nodule (see Fig. 6) showing characteristic pseudomorphous calcareous algal deposits, × 2.3.

Fig. 8. Conglomerated rounded nodules from the Greenfield dolomite, × 0.4, (O. S. U. Geol. Mus. No. 9868).

Fig. 9. Shingled flat nodules from the Greenfield dolomite, viewed in plane of bedding, × 0.66.

Fig. 10. Section of right end of specimen in preceding figure, viewed across bedding plane, × 1.

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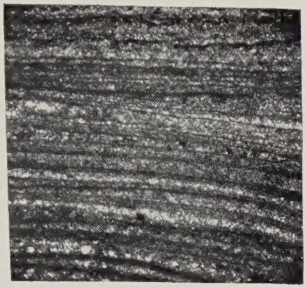

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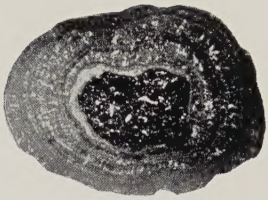

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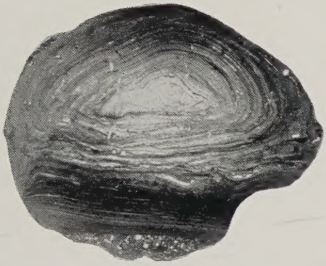

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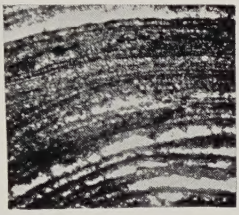

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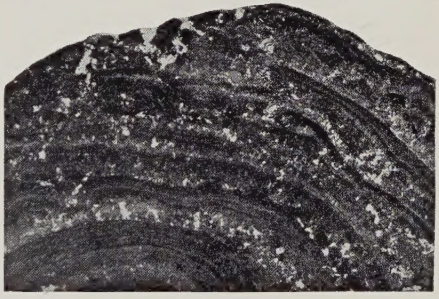

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porostromatids as *Sphaerocodium*, *Ortonella*, *Mitcheldeania*, and *Girvanella*, nor the close-packed prismatic tubules of the rhodophycean *Solenopora*, nor the undulant spongy microcrystalline layers of less determinable pseudomorphous forms with faint tubular molds of filamentous algae such as the recent "water biscuits" of Canandaigua Lake and other localities, the "ooids" described by Eardley[9] from the Flagstaff formation (Eocene) of Utah, and the nodules of *Zonotrochites* from the European Rhaetic figured by Bornemann[10]. Thin sections of nodules illustrative of this pseudomorphous type (*Zonotrichites*) are figured on Plate I, Figs. 5–7[11].

Nor are the Greenfield nodules like some of the less authentic, but none-the-less probable algal structures which formed similar masses,—the "stromatoliths," such as the famous *Cryptozoon* of the Cambrian and Ordovician, some specimens of which, however, do show faint traces of tubular molds[12], and *Ottonosia*[13], from the Pennsylvanian of the mid-continent area, in which there may be tubular molds and in which the nodular form of the sharply-defined but very irregularly discontinuous laminae does not smooth out upwards.

Against these evidences of lack of correspondence with known algal structures, we have only the point that the laminae of the Greenfield nodules do not show the radial fibrous structure of physico-chemical encrustations, but such structure, if ever present, may well have been obliterated by the subsequent dolomitization. On the other hand, the uniformity of the laminations, both laterally and vertically, as contrasted with the irregular and undulant laminations[14] of genuine algal deposits, the absence of definite calcareous algal structures such as tubules, and the lack of pseudomorphous spongy layers with tubular molds, are all suggestive of inorganic deposition.

CONCLUSION

The nodules found in the Greenfield dolomite at its type locality near Greenfield, Ohio, show no traces of structures attributable either directly or indirectly to algae and are thought to be the result of physico-chemical deposition.

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[9]Eardley, A. J., 1932, "A Limestone Chiefly of Algal Origin in the Wasatch Conglomerate, Southern Wasatch Mountains, Utah." *Papers Michigan Acad. Sci., Arts, and Letters*, Vol. 16, pl. 32.

[10]Borneman, J. G., 1887, "Geologische Algenstudien," *Jahrb. Preuss. Geol. Landes, für* 1886, pl. 5, fig. 1.

[11]Specimens from Upper Cretaceous fresh-water deposits in Utah, collected by Dr. E. M. Spieker, to whom the writer is indebted not only for these specimens but also for others from the Upper Jurassic and the Eocene of the same state.

[12]Goldring, W., 1938, "Algal Barrier Reefs in the Lower Ozarkian of New York . . ." *N. Y. State Mus. Bull.* No. 315, fig. 14 *et al.*

[13]Twenhofel, W. H., 1919, "Pre-Cambrian and Carboniferous Algal Deposits," *Amer. Jour. Sci.*, Vol. 198, p. 348.

[14]Bradley (*op. cit.*, p. 218) states in connection with the laminated algal deposits in the Green River formation that "they are arcuate [and] considerably different from the fibrous incrustation of acicular crystals, . . . much less uniform, . . . vary rapidly in thickness from place to place, and their upper surfaces are very irregular and serrate, not smooth like the layers in agate structure."

COMPOSITION OF FUNGUS HYPHAE

III. The Pythiaceae[1]

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The genus *Pythium*, established by Pringsheim in 1858, has long been recognized as an interesting group of fungi (1). The economic importance of this genus was first indicated by Hesse in 1874. Since that time appreciation of the importance of this group has increased as agricultural production has become more intensified.

Interest in the Pythiaceae, aside from their economic importance as damping-off fungi, has been chiefly from the standpoint of morphology and cytology. Very little is known regarding the nature of the hyphae or the by-products of metabolic activity. This report is concerned with the composition of the mycelium. It has been the purpose of this investigation to determine the nature of the substances occurring in the mycelium, to identify them, and to learn how they are arranged.

SPECIES INVESTIGATED

The type species, viz., *P. monospermum* and *P. entophylum*, used by Pringsheim in establishing the genus *Pythium* could not be obtained. In this study, six identified species of Pythium obtained from the American Type Culture Collection, and three unidentified isolates were investigated. The group of known species included *P. debaryanum* Hesse, *P. vexans* deBary, *P. ultimum* Trow, *P. acanthicum* Drechsl., *P. aphanidermatum* (Eds.) Fitz., *P. spinosum* Sawada, and three species isolated, respectively, from diseased tomato seedlings, begonia, and potato.

PLAN OF STUDY

Cultures were developed in 300-cc. flasks containing 125 cc. of potato broth filtered free of all sediment. This medium was found to be very satisfactory as well as easy to prepare in quantity. Richard's solution with saccharose as the carbohydrate permitted only meager growth. With glucose substituted for saccharose, development was much better, and the addition of 0.1 per cent of yeast extract still further improved the quality of the medium for *Pythium*. Although the modified synthetic solution was used in the first part of the work, it was soon replaced with potato broth, which was more satisfactory.

Investigation of the mycelium of each species was made at different periods of growth, varying from 3 days to 3 months. For the most part, only fresh cultures were used. When it was not possible to complete the analysis at a definite stage of development in the time available, the mats were washed free of culture solution and preserved in 95 per cent alcohol until required for use. This was found to be particularly important with *Pythium*, because a growth in a favorable medium, the first few days, is rapid.

Both microchemical and macrochemical methods were employed. Only small quantities of hyphae were used in many of the tests: the amounts were, in most cases, equivalent to less than 0.05 gm. of dried mycelium. The general procedure consisted in the detection, removal, and identification of the different constituents of which the hypha is made up. Appropriate physical and chemical tests and solvents were used. The technique was based upon procedure described by Molisch (4), Tunmann (8), Klein (2), Morrow (5) and Campbell (9).

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[1]Published with the approval of the Director of the Ohio Agricultural Experiment Station.

Examination of young vigorously growing hyphae 2 to 3 days old showed that the material on the outside is not anisotropic or doubly refractive. This property gradually changed. After 5 to 7 days of growth, at 25 degrees C., the older portions of the hyphae become dimly anisotropic; and as the culture advanced in age, this physical property became more evident, yet the young, newly formed, hyphal tips were still found to be dark when viewed in polarized light. Old cultures that had ceased activity after 3 months of growth were anisotropic throughout the entire extent of the mycelial filaments.

When very young hyphae were immersed in a weak solution of ruthenium oxychloride, the dye became fixed in the walls and could not be washed out with water. This is a presumptive test for pectic compounds. For futher confirmation of the presence of pectic material, several mats, washed free from culture liquid, were then placed in 0.5 per cent aqueous solution of NaOH and allowed to stand for several hours with occasional shaking. The alkali not only dissolved the pectic substance from the hyphae, but also changed it to pectin. The liquid was filtered and divided into two portions. One portion was rendered acid with HCl, and to the other was added an excess of $CaCl_2$. A gelatinous precipitate occurred in both instances. The first was pectic acid, the second, calcium pectate.

The precipitates were collected by centrifuging and portions tested with the orcinol-HCl reactions. A color change occurred ranging from pink to violet and blue or blue-green. When a trace of $FeCl_3$ was added after the pink color had developed, the transition to green took place promptly. In all cases the color could be removed by shaking with amylic alcohol.

After removal of the pectic material, the hyphae were found to be doubly refractive even in young cultures. Other cell wall substances of fungi which are known to possess this property are callose, and cellulose. Chitin of lobster carapace is anisotropic en masse, but when it occurs in fibers or strands this characteristic is less conspicuous.

Very young cultures which had been rendered free of pectic compounds by aqueous alkali, and old cultures in which the pectic material had been decomposed by autolysis did not fix dyes. This was anticipated, because previous investigations of the *Fusaria* and *Sclerotinia* groups by the author (6, 7) had demonstrated the lack of affinity of the hyphae of those groups for stains. This lack was found to be due to the inert character of the fatty acids with which the mycelium was impregnated. When the hyphae at this stage of the analysis were treated with 1 per cent alcoholic potash by refluxing, the liquid at first became yellow in color, deepening in intensity until a maximum was reached. After the hyphae had been washed in alcohol until all alkali was removed, treatment with 1 per cent aqueous potash did not separate any material. Callose would be soluble in aqueous potash after the fatty acids had been saponified and dissolved from the hyphae (7). Treatment with bromine water for 24 hours followed by 1 per cent aqueous alkali also failed to render any cell wall substance soluble.

With the probability of the presence of callose eliminated, attention was now directed to the detection and identification of cellulose, since the hyphae were more strongly doubly refractive after the removal of fatty acids and fixed such aniline dyes as methylene blue, congo red, and safranin.

A test for cellulose was next made. Lugol's iodine solution followed by 70 per cent sulfuric acid caused the mycelium to develop a blue color, which was indicative of cellulose. Old, partially autolyzed cultures demonstrated a blue color with the same test, yet much more quickly after previous treatment with alcoholic potash to remove fatty acids. Young cultures, 3 to 10 days old, without any other treatment, showed only the yellow or brown color of the iodine, and no blue absorption compound characteristic of cellulose developed with the addition of sulfuric acid.

Cellulose is soluble in ammoniacal cupric hydrate. This reagent had no solvent action upon young hyphae less than a week old; yet, when mycelium of the same age, with pectic material and fatty acids removed, was subjected to the reagent, cellulose passed into solution. This was demonstrated by filtering off the liquid through a glass filter, diluting the filtrate with several volumes of water, and acidifying with acetic or hydrochloric acid. With this treatment, cellulose was precipitated. This precipitate was doubly refractive in polarized light, gave a blue color with iodine and sulfuric acid, and upon hydrolysis, yielded a reducing sugar from which a phenylosazone was prepared which was found to be identical in appearance and melting point with the dextrosozone.

The same result was accomplished by hydrolyzing the cellulose with 70 per cent sulfuric acid. Both mycelium and acid were cooled in a refrigerator to 8 degrees C. before the mycelium was placed in the acid. After 12 hours sufficient water was added to reduce the strength of the acid to 3.5 per cent, and hydrolysis was completed by refluxing.

The solid residue in both cases, washed free of the reagents, no longer gave a test for cellulose, was no longer doubly refractive in polarized light, and did not fix aniline dyes.

This residue was tested for chitin. It was boiled for 30 minutes in saturated KOH solution, then washed and hardened in 90 per cent alcohol. A red violet color developed when the substance was placed in Lugol's iodine solution diluted with 2 parts of water. If too strong an iodine solution is used, the color change may take place so rapidly that the violet color may appear black. In addition to the chitosan reaction, chitosan sphaerites and glucosamine hydrochloride were prepared. Thus, the residual portion of the *Pythium* complex was identified as chitin.

SUMMARY

An analysis has been made of the mycelium of six different species of *Pythium*, and the same composition has been revealed in all the species investigated.

The outer covering of young hyphae was found to be pectic material. This covering could be removed by suitable solvents to disclose underneath a layer of cellulose. The pectic substance on the outside of young hyphae completely masked the cellulose and prevented reagents from reaching it. Old, mature hyphae are doubly refractive to polarized light and, therefore, devoid of pectic substance on the outside.

The cellulose was strongly impregnated with fatty acids which prevented the fixation of dyes. The fatty acids could be removed by saponifying with alcoholic potash.

The residual or basic skeleton of the Pythium mycelium was identified as chitin.

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A MAP OF THE WORLD IN PERSPECTIVE[1]

A New Attempt to Improve World Areal Plottings, Based Upon Eckert's No. 6 Projection[2]

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In representing the earth's spherical surface upon a plane, any map projection must inevitably involve some distortion, which increases with the size of the area included. A world projection, developing the grid of the entire earth, therefore, presents maximum difficulty in overcoming distortions. The principal properties which may be preserved on the map, as they are on the earth, may be listed as follows: (1) equivalence, or true relative sizes of areas; (2) conformality or true angles formed by intersecting lines (compass directions, if grid lines are straight, as on the Mercator projection); (3) true shapes of areas; (4) equidistance, or true scale, along certain lines; (5) continuity of surface, with the grid unbroken.

Preservation of all of these at once is possible only on the globe. On a flat surface one or several of the properties may be kept, but there is always a sacrifice. Evaluation of a projection for any purpose thus involves a balancing of the gain with the loss. Some projections which develop special traits lose all of the above properties. This is a high price, but it may be worth it, as is the case with the invaluable gnomonic projection, on which a great-circle route is easily determined from a straight line. The Mercator map preserves true compass bearings throughout, and so is indispensable for correctly plotting any directional data. Yet neither of these navigation charts should be employed for any use where areal relationships

[1]The figurative rather than literal interpretation of this word is intended, for obviously the entire earth surface cannot be shown in true perspective. The orthographic hemispherical projection is the only true perspective representation.

[2]For a summary and illustrations of Eckert's series of six projections, cf. Max Eckert, "Neue Entwürfe für Erdkarten," Petermanns *Mitteilungen*, Vol. 52, pp. 97–109, 1906. The writer is indebted to Mr. John B. Leighly, University of California, for first calling attention to Eckert's projections, and for providing the necessary grid data.

are desired, since sizes, shapes, and scales are badly distorted away from the tangency. Nevertheless, the Mercator projection is today probably the most widely employed base for world plottings which depict areas and distributions. This despite the years of effort on the part of geographers to supplant it with equivalent projections.

These attempts may have failed because of an over-emphasis upon the value of shape. The map herewith presented[3] was produced on this premise, and represents a new approach towards the establishment of a suitable equivalent map. desirable now as never before in view of unprecedented interests in world areas, It is assumed, then, that the major properties listed above may be arranged as follows in descending order of importance upon a world areal map: (1) true sizes of areas, (2) continuity of grid, (3) true bearings of grid lines, (4) true shapes of areas, (5) true scale. It is impossible to preserve either of the last two, namely, true shapes and scales, on all parts of any world map. And only the Mercator projection shows true bearings throughout.

An appraisal of the "perspective" map, illustrated in this paper, may be made in terms of these five properties. First of all, it is equivalent, with no size distortions. Secondly, it is plotted on an unbroken grid. There is less attempt to preserve true continental shapes, as is often approached on world maps at the expense of the ocean areas,[4] than to maintain continuity of continents, not only upon the grid, by centering the meridian of 10° east longitude in order to split the Pacific through the Bering Strait and thus keep the continents entire, but also beyond the grid by lateral extensions which repeat northwestern North America and northeastern Eurasia so as to bring out the essential relationships of the lands bordering the North Pacific.[5] As to the third point, east and west are true directions, since the parallels are straight. This keeps the important factor of latitude on a comparative basis, as is not the case where parallels are curved. Though there is inevitably a deviation from true north and south, since meridians are curved, it is not nearly so great as on the more compressed elliptical projections, such as the Homolographic. For this latter reason, the fourth property, namely shape, is less subject to distortion than on the elliptical and "semi-elliptical" projections, though they may not even preserve equivalence. That shapes of marginal areas are distorted on Eckert's No. 6, cannot be denied, yet this does not seem to be too high a price to pay for the areal relationships which it brings out. The sine curves, from which the term "semi-sinusoidal" may be applied to the projection, maintain the high-latitude grid spaces more open than is possible with elliptical meridians. Finally, scale, though exactly true only along the equator, is closely approximated along all grid lines out to the fortieth parallels. By stating scale in terms of miles between grid intervals rather than miles to the inch, errors are avoided even in high latitudes. A further advantage of this type of projection is the manner in which it suggests the spherical form of the earth, maintaining something of the true position of the "down-under" lands of the southern hemisphere, such as Australia. This is lost on the Mercator and on most of the interrupted projections.

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[3]Published in outline as "McBryde's Perspective Equal-Area Base-Map," College Book Co., Columbus, Ohio.

[4]The "interrupted" projections, e. g., give a false appearance of exaggerated width to the Atlantic, and the "jig-saw puzzle" maps cut out the oceans and fit the continents snugly together in wholly misleading juxtaposition.

[5]Such lateral repetition is not practicable on projections where meridians are ellipses, and on the cylindrical projections, such as Mercator's, it may be done only at the expense of considerable space, for the entire set of meridians must be repeated. Thus, the "semi-sinusoidal" projection is the only one on which the extensions may be satisfactorily fitted into unoccupied corners of the map off the grid.

AN EFFICIENT VACUUM APPARATUS FOR MICROTECHNIC

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From time to time notes on vacuum apparatus and its applications to microtechnic have been published. These papers, with the exception of very few instances, do not deal with the actual measurement and control of vacuum, the effect of vacuum on tissues and the pumping of tissues in reagents not miscible with water and water vapor. It seemed necessary, therefore, to check the above conditions and to determine, if possible, their relation to fixation, dehydration and infiltration of paraffin in plant material in the "in vacuo" process.

Lebowich (4) in 1936 developed a soap-wax medium for the simultaneous dehydration and infiltration of human tissues. In this technic he employed a vacuum apparatus consisting of a faucet aspirator and a wide-mouthed bottle placed in an oven automatically controlled for temperature.

Later Moritz (5) modified Lebowich's vacuum pump. Instead of using the expensive equipment which Lebowich had at his disposal, Moritz attained the same end with ordinary laboratory equipment obtainable by any technician. In the same paper he presented a modification of Lebowich's dehydration schedule. In the technics of both of these men a vacuum apparatus was used to definite advantage in regard to time and thoroughness of preparation of tissues.

In April 1940 Chermock and Hance (2) announced the use of a vacuum pump for general micrology. Their principle dehydration agent was "methyl cellosolve" which they used in a vacuum apparatus of simple and convenient construction. Their accessory devices were a thermometer and a water trap.

Johansen (3) illustrated a pump which he uses to extract the air from plant tissues in the process of fixation.

Sass (6) described a pump for speeding up killing and fixing of material. This apparatus again consists of the usual essentials of a vacuum pump with a finger valve for the manipulation of a slight increase or decrease of vacuum within the specimen bottle.

It must be remembered, however, that the less complicated vacuum systems used by many of these men were sufficient for their needs as they were not used for the complete processes from fixation through paraffin infiltration but were used only for one or another of these processes.

The vacuum pump is becoming more and more a necessary piece of equipment in microtechnical problems (Fig. 1). Before the initiation of the present pump system a faucet aspirator was used to which was attached a length of pressure tubing. A rubber stopper sealed this tubing directly to the individual specimen bottle. Most material pumped by this method, with the exception of some more woody stems, showed, when cut, one or more types of injury due to the extreme increase or decrease of vacuum. Some of these injuries were wavy cell walls, splitting of cell walls along the middle lamellae, abnormal vacuolization of the cytoplasm, absence of portions of the secondary walls, the crushed appearance of phloem cells, and the tendency of epidermal and peridermal tissues to break away from cortical tissues. At first these injuries were thought to be due to insufficient dehydration, insufficient infiltration or overheating while the material was in

[1]Papers from the Department of Botany, Ohio State University, No. 434.

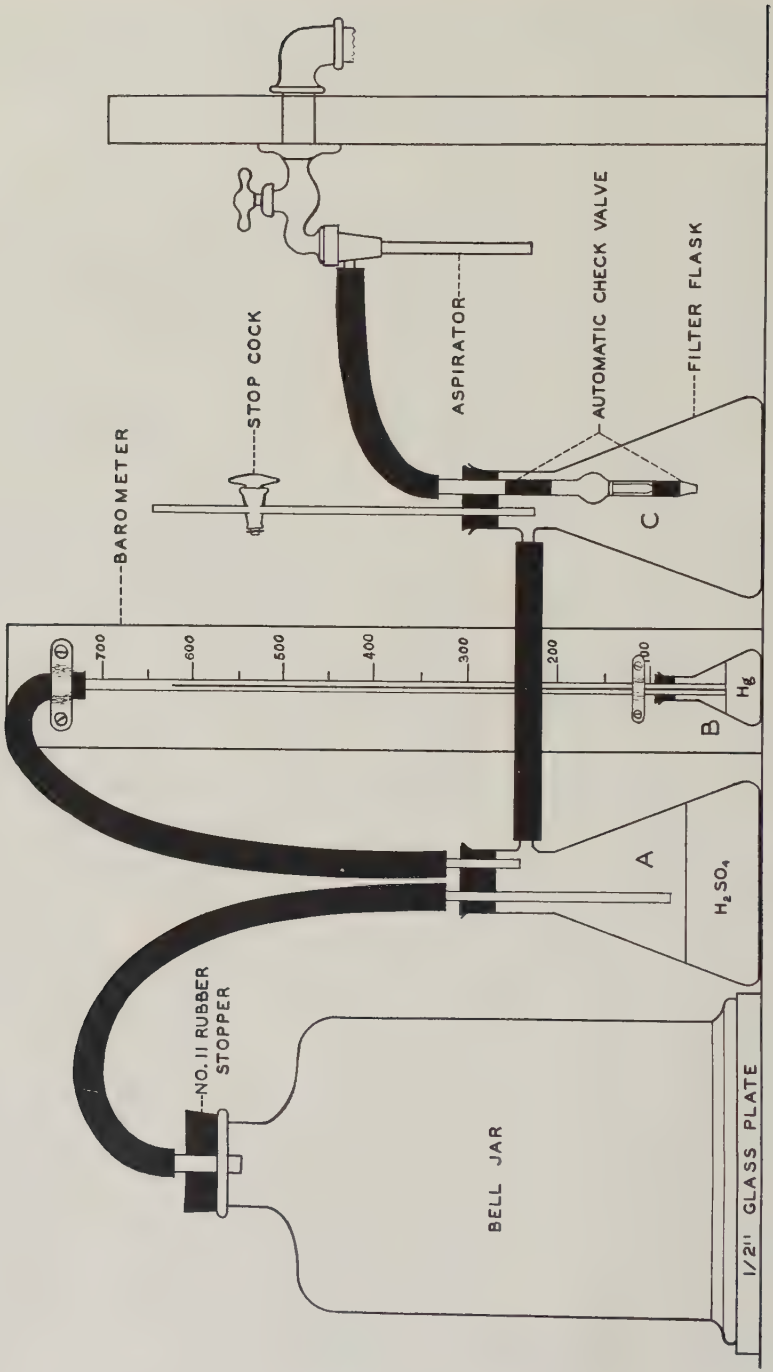

Fig. 1. Vacuum apparatus

paraffin. It was only when endeavoring to find the source of these injuries, that it was discovered that the very construction of the pumping system made it impossible to control the vacuum properly. Later, when the displacement of air was measured, the data showed a displacement rate of over 400 mm. mercury in one minute. This is a tremendous change of pressure vs. the pressure within the plant cell. As a result large amounts of air in the tissues rip through intercellular spaces and plant walls of softer tissues, rendering specimen material useless for a good finished preparation. Therefore, a pump system was constructed which would make impossible a rapid displacement of air and enable the operator to maintain a given displacement of air over a period of time. As a result of several experiments the present pump system evolved. The entire process of fixation, dehydration, and infiltration of paraffin were carried out under vacuum.

Some time ago Dr. D. M. Crooks devised an automatic check valve for a vacuum pump which has been in use at the University of Arizona. By means of this valve a critical control of the vacuum apparatus can be maintained. Although this valve has been modified slightly, the principle of operation is the same and the details of the valve are shown in the diagram (Fig. 2).

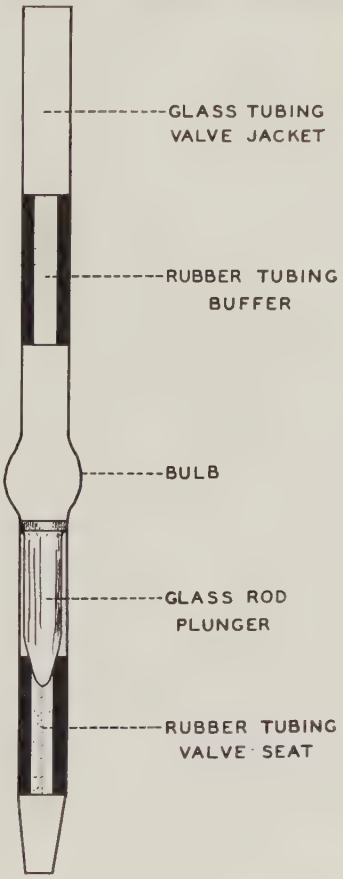

Fig. 2. Automatic check valve

To facilitate the rapid passage of air around the plunger of the valve, it was necessary to blow a bulb in the valve jacket. This bulb prevents the plunger from pushing the rubber tubing buffer out of the jacket. When constructing the glass tubing valve jacket, close one end by drawing it out with a twirling motion over a Bunsen burner. Next, heat evenly a section of the glass tubing one inch in length and three inches from the closed end. Continue the heating until the glass attains an even red glow, draw the tube away from the flame and immediately blow through the open end with quick, short breaths until the bulb is approximately one-half inch or more in diameter. A little practice may be necessary before an evenly blown bulb can be obtained. The closed end of the glass tubing should be cut where its diameter approximates three millimeters. In completing the jacket glaze the cut end until the opening is one or two millimeters in diameter. Another step in the construction of the valve is the insertion of the rubber tubing valve seat and buffer. First, cut two pieces of rubber tubing as specified in footnote 2, one and one-fourth inches in length. Moisten one piece and insert into the valve jacket. Then, with a glass rod which is slightly less in diameter than the inside diameter of the jacket, push the rubber tubing into the position shown for the valve seat as in Figure 2. Next, insert the glass plunger and push the second piece of moistened rubber tubing into the position shown for the buffer in Figure 2.

The list of materials mentioned below was used in the construction of the vacuum apparatus.[2] One or several bell jars may be used. The larger the capacity of the bell jar the better since the rate of increase of the vacuum is thereby reduced. These may be attached to the pump system by inserting one or more glass "Y" tubes in the exhaust line leading from the sulphuric acid flask (Fig. 1, A). With this arrangement a stop cock (Fig. 1) inserted on each bell jar and a hand check valve on the line leading to each bell jar enable the operator to remove the contents of one container without disturbing the others. This is a very worthwhile suggestion made by Dr. Crooks.

Aside from constructing the automatic check valve, the operation of the pump is probably of equal importance. If the faucet to which the aspirator is attached is turned wide open when using a 25 liter bell jar, the mercury column will rise approximately 250 millimeters the first minute, 150 millimeters the second minute and gradually decreases in rate each succeeding minute until a maximum of 700–720 mm. mercury on the barometer column is reached. It was found that the first two or three minutes of air displacement causes the damage to plant material. If the operator will reduce the excessive increase of air displacement during the first few minutes to not more than 150 mm. mercury per minute, the danger of damaging plant material is appreciably lessened.

All dehydration schedules tried were found to be easily cut in half with the aid of the pump. One reason for this is that diffusion in a vacuum takes place much more rapidly than under atmospheric pressure. Also, there is no air in the tissues to hinder diffusion. In a dehydration schedule where changes of reagents were made at two hour intervals, the change of the material under the pump was made at one hour intervals with exactly the same results.

Above 600–650 mm. mercury some damage of specimen material occurred. Just as important is the gradual reduction of the vacuum. The best results were obtained when the reduction did not exceed 90 mm. mercury per minute.

When the bottom flange of the bell jar (Fig. 1) was not vaselined carefully or vaselined at all, the decrease of the vacuum was allowed to take its own course through leakage. When a tight seal was maintained, it was necessary to reduce by opening the stop cock (Fig. 1).

A question might be raised as to the purpose of two pyrex filtering flasks in the pump system. The first (Fig. 1, C) was installed so that the automatic check valve could be held vertically and yet firmly in place. The second flask (Fig. 1, A) containing sulphuric acid was introduced to absorb most of the water vapor. The acid prevents the clouding of toluene and xylene which occurred before in dehydration steps in which these liquids were used.

In the process of paraffin infiltration the exhaust tubing which was originally attached to the bell jar was inserted through the side vent of a standard constant temperature oven. A number 11 rubber stopper was attached to a wide-mouthed 500 cc. bottle within the oven. Greater care in the operation of the pump should be exercised since a much smaller vacuum chamber is being used. The maximum millimeters of mercury should not exceed 400. The rate of increase or decrease of vacuum per minute was adhered to as previously mentioned. Five changes of

[2]One standard faucet aspirator, two 1000 ml. Erlenmeyer pyrex filtering flasks with side necks, one 30 ml. Erlenmeyer flask, one 18–36 liter bell jar with open top and ground glass bottom flange, one piece of ½ inch glass plate 16 inches sq., one glass stop cock with a 1 mm. bore, one piece of barometer tubing 3 ft. long with a 2 mm. bore, one piece of glass tubing 14 inches long with an outside measurement of 12 mm. and an inside measurement of 9 mm. for valve jacket, one piece of 7 mm. glass tubing 3 ft. long for connections, one piece of 8 mm. glass rod 5 inches long for the 1½ inch valve plunger, one No. 11 rubber stopper, two No. 8 rubber stoppers, one piece of 10 mm. black gum rubber tubing 2½ inches long for valve seat and buffer, and 6 ft. of rubber pressure tubing.

paraffin were used at intervals of three hours (25%, 50%, 75%, and two changes of 100% paraffin) before imbedding. An automatic temperature control oven regulated to oscillate between 52° C. and 58° C. resulted in the temperature in the vacuum bottle oscillating between 54° C. and 56° C. By this control the danger of overheating material was eliminated. A slight improvement as to the smoothness of texture of the paraffin was also observed.

Beside the actual use of the pump for the processes mentioned above, it is extremely useful when preparing demonstrations of the vascular system of the entire plant. *Coleus* plants cut off at one of the lower internodes and placed in an aqueous solution of basic fuchsin according to Camp and Liming's method (1) take from three to five days to clear properly in xylene. The clearing of these demonstration stems is a matter of several hours if the vacuum apparatus is used. This apparatus also permits the fixation of as much as three liters of plant material in a few hours. In permanent prestaining schedules, tissues pumped in the dye, stain very rapidly. The maceration of woody stems such as *Quercus*, *Liriodendron*, *Pinus*, and many others was obtained in one-half to one-third of the normal time otherwise required.

The following list of plant materials was used in this experiment: the roots, young and old, of *Liriodendron*, *Ranunculus*, nine species of the *Amaryllidaceae*, *Raphanus*, *Citrullus*, *Coleus*, and *Daucus carota;* the stems of *Helianthus*, *Zea mays*, *Vinca minor*, *Carica papaya*, *Echinochloa*, *Elymus*, *Pinus strobus*, *Hamamelis virginiana*, *Benzoin aestivale*, *Trifolium*, *Avena*, *Triticum*, *Phleum pratense*, *Picea;* the leaves of *Amaryllis*, *Hymenocallis*, *Trifolium*, *Aesculus glabra*, *Ranunculus serratus*, *Sarracenia purpurea;* the seed or flower parts of *Triticum*, *Coleus*, *Hymenocallis*, *Aesculus glabra* and *Ranunculus serratus*. The fungi used were *Poria*, *Polyporus*, *Coprinus*, and *Scleroderma*. The algae used included the genera *Sargassum*, *Fucus*, *Ulva* and *Chondrus*.

SUMMARY

1. The vacuum apparatus is a necessary piece of equipment for critical results.

2. The time element is cut to one-half normal time for most dehydration and infiltration schedules.

3. Air in the tissues is completely removed and this in turn accelerates diffusion.

4. Many difficulties in paraffin infiltration, such as temperature fluctuations, overheating and partial removal of air, are to a great degree eliminated.

5. A large amount of material may be carried through a given schedule at one time.

6. With this apparatus the factors affecting material while in a vacuum are more readily controlled by the operator.

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A NEW SPECIES OF SPIROGYRA

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and
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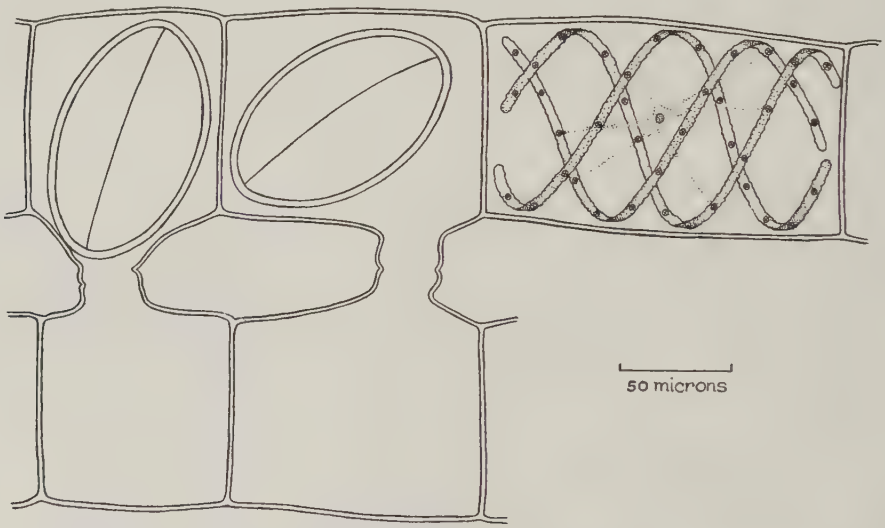
Indiana State Teachers College

Spirogyra hymerae n. sp.

Vegetative cells 83–92 x 43–256μ, with plane end walls; 2–4 chromatophores, usually 3, making 0.5–2 turns in the cell; conjugation scalariform, tubes formed by both cells; receptive gametangia cylindric or slightly enlarged; zygospores mostly ellipsoid, 53–79μ x 83–128μ, all walls smooth, median wall yellow.

Cellulis vegetativis 83–92 x 43–256μ, dissepimentis planis; chromatophoris 2–4, anfractibus 0.5–2; conjugatione scalari; tubo ex utraque cellula conjuganda emisso; cellulis fructiferis cylindricis, vel paululum inflatis; zygosporis ellipsoideis 53–79μ x 83–128μ, mesosporio laevi, flavescente.



To be compared with *Spirogyra setformis* (Roth) Kuetzing from which it differs in number of chromatophores and smaller size.

Collected by the authors from a small pond at Hymera, Sullivan County, Indiana, September 10, 1936.

Type material has been distributed in the following herbaria: (1) Dr. E. N. Transeau, The Ohio State University, Columbus, Ohio; (2) Cryptogamic Herbarium of the Field Museum, Chicago, Illinois; (3) the authors' (Ind. No. 32). The authors wish to express their thanks to Dr. E. N. Transeau for his aid in making proper disposition of this species.

THE LENGTH OF THE PHOTOPERIODICALLY EFFECTIVE TWILIGHT PERIOD[1]

VICTOR A. GREULACH

The results of studies of critical photoperiods emphasize the importance of knowing the exact length of any photoperiods employed experimentally. Since most experiments are at present conducted under photoperiods artificially regulated in length this information is usually available. However, in the past the natural photoperiod has frequently been employed for either long or short days, and even at present it is sometimes necessary to employ natural photoperiods experimentally, especially in field studies. Little attention has been given to accurate determination of the lengths of the natural photoperiods, the time from sunrise to sunset usually having been taken as the length of the photoperiod. In only a few papers, such as one by Forster, Tincker, Vasey and Wadham (3) and one by Magruder and Allard (4), has the twilight period been considered.

In view of the results of studies on the minimum light intensities which are effective photoperiodically, it would appear that the naturally occurring effective photoperiod is always somewhat longer than from sunrise to sunset. Austin (1) found the minimum photoperiodically effective light intensity for *Rudbeckia bicolor* and *Cosmos sulphureus* to be between 0.4 and 1.8 foot candles. Borthwick and Parker (2) found the light intensity necessary to prevent induction of Biloxi soy beans to be between 0.5 and 1.0 foot candle. Similar ranges have been found in other species which have been studied. Obviously, such light intensities are attained some time before sunrise and persist until some time after sunset.

In an effort to secure some information on the length of the photoperiodically effective twilight period the writer conducted six series of light intensity measurements, beginning before sunset and continuing until a photronic foot candle meter (Weston, No. 614) graduated to one foot candle failed to deflect. Two similar series of readings were made in the morning, beginning as soon as the scale was visible and extending until after sunrise. Readings were made at frequent intervals, in order to determine the time at which the light intensity was 1 foot candle. This was arbitrarily considered to be the minimum photoperiodically effective light intensity, since the actual minima for species studied do not vary greatly from this. The measurements of light intensity were made in an open portion of the Botanical Gardens at the Ohio State University in Columbus, Ohio 40° N.). Measurements were made at normal incidence, *i. e.*, with the face of the cell at right angles to the direction of the sun's rays, or, before sunrise and after sunset, as near normal incidence as possible. The data secured are presented in Table I.

Assuming 1 foot candle as the minimum light intensity which is photoperiodically effective, the lengths of the effective twilight periods measured varied from 11 to 28 minutes, the mean being 19 minutes. The presence of clouds shortened the length of the photoperiodically effective twilight period materially, the mean being 17 minutes for cloudy days and 26.5 minutes for clear days. The range of effective twilight for this latitude is therefore from about 10 minutes on very cloudy days to about 30 minutes on clear days. Since both the morning and evening twilight periods must be considered, the photoperiodically effective twilight may range from about 20 minutes on cloudy days to about an hour on clear days.

[1]Papers from the department of Botany, The Ohio State University, No. 432.

Since the critical light intensity for some species is less than 1 foot candle these limits might be extended somewhat, but not greatly, as the failure of the needle of the photometer to deflect occurred on the average only 4 minutes after (or before) the 1 foot candle point was attained. On the other hand, even if the minimum effective light intensity is 2 foot candles the length of the effective twilight would not be shortened by more than a few minutes, due to the rapid change in light intensity just before sunrise and just after sunset.

TABLE I

LENGTH OF THE PHOTOPERIODICALLY EFFECTIVE TWILIGHT PERIODS ON SELECTED DAYS

Date (1939)	Sky Condition	Time of Sunrise (R) or Sunset (S)	Time when Light Intensity was 1 Foot Candle	Minutes of Effective Twilight
July 6	Cloudy	R. 5:06 A. M.	4:48 A. M.	18
July 19	Cloudy	R. 5:15	5:00	15
July 5	Cloudy	S. 8:07 P. M.	8:27 P. M.	20
July 11	Clear	S. 8:05	8:30	25
July 12	Scattered clouds	S. 8:04	8:25	21
July 13	Clouds over sun	S. 8:04	8:15	11
Aug. 10	Clear	S. 7:38	8:00	28
Aug. 19	Cloudy	S. 7:28	7:45	17

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Magic in a Bottle

This fascinating book tells the stories of the discoveries of important drugs and medicines and their discoverers. The author is a professional journalist and holds a Ph.D. in pharmacology, so thoroughly knows his subject and how to relate it. His style of writing reminds one of Paul DeKruif. The biographies of the discoveries of the drugs are dramatized to such an extent that they seem more like adventure fiction than like histories from one field of scientific achievement. The following excerpts from the foreword are samples of the type of writing encountered throughout the book. "Great-great-grandfather swallowed a concoction of colchicum and fennel water and Bavarian tar, well mixed, with sassafras and oil of wintergreen and powdered opium or rue. That brew didn't do him much good for he died in spite of it—or maybe because of it. Today my daughter takes one milligram of 3^1 (methyl-4-amino-hydrochloride-pyrimidyl-5-methyl-N-4^1-methyl-5-beta-hydroxy-ethyl-3^1-chlorothiazole), and she stays strong and healthy. . . . The twentieth-century doctor protects my little girl with a single magic bullet, aimed straight at the exact devil that tries to harm her and that's why all the statisticians will bet their shirts she'll live for another sixty years.

The book should make especially interesting reading for high school and college students interested in science.—*D. C. Rife.*

"Magic in a Bottle," by Max Silverman. X Chap., 323 pp. The MacMillan Co., 1941. **$2.50.**

SOME NEW SPECIES OF BACCHA AND MESOGRAMMA

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In this paper I present the description of three new Syrphid flies from South America. Types, unless otherwise stated are in the collection of the author.

Baccha brunnipennis n. sp.

Related to *B. lepida* Macquart but the wings much darker, the hind femora and tibia wholly dark brown besides abdominal pattern differences.

Male.—Length 8 mm. *Head:* vertex blackish-brown; the front is broadly, dully, brownish-black; the sides of the front continuously light orange-brown. The face is pale yellowish-brown, the middle of the face is deeper yellow, the facial pile is brown with some pale hairs intermixed; the cheeks very small and yellow. The first and second joints of the antennae are light brown; the third joint is light reddish-brown with the dorsal margin and arista quite dark brown. *Thorax:* mesonotum shining dark brown with a metallic, slightly brassy luster and two brown pollinose vittae, one on either side, and an additional pair on the lateral margin, and a very slender, similar, linear streak down the middle. The dorsal pile of the mesonotum is brownish black. Pleurae metallic black with a bluish cast, the black not enclosing the posterior spiracle; almost the whole of the mesopleurae, upper part of sternopleurae and anterior part of pteropleurae, light, translucent brownish-yellow. The scutellum is brownish-yellow with only a very few dark hairs and with the ventral fringe apparently non-existent. *Abdomen:* spatulate, but a little wider at apex than at base and marked with brownish-yellow as follows: just past the middle is a slightly diagonal, sublateral fascia connected in the midline though there is trace of an interruption; and upon the third segment a thin, basal, submedial, posteriorly rounded fascia that ends some distance before the lateral margin and in the middle large triangles well separated, not quite reaching the sides, whose right-angular angle is posteromedially placed. On the third segment there are well separated large yellowish V's, one on either side and inverted, the ends rounded and the anteriorly directed apex narrowly confluent with the submedial, basal, yellow spots which are somewhat similar to those of the proceeding segment but are a little larger. The arms of the V-shaped figure are broad and thick. Last segment with four vittae, the median pair somewhat longer, none of them reaching the posterior or lateral margins and the pair on each lateral half is confluent along the basal margin of the segment. *Legs:* first pair of legs almost wholly light brownish-yellow, the coxae palest, the base of their femora slightly smoky, their pile chiefly pale but some of its femoral pile dark. Middle pair of legs somewhat similar to the first pair, their femora darker and more extensively brown, the apex still pale and the posterior surface with a prominent fringe of long brown pile. The hind femora and their tibiae are very dark brown with brownish-black pile. Only the apex of the former is lighter brown. The hind tarsi, which are quite elongate and brownish-orange, are slightly darker towards the base of the basi tarsi, their pile brown. *Wings:* wholly dark sepia throughout, moderately broad, the allula narrow and strap-shaped.

Holotype: one male, Sao Paulo, April 6, 1939, J. Lane, collector; one paratype, same locality, in the collection of Mr. John Lane.

Baccha beatricea n. sp.

Related to *B. clarapex* Wied. but with apical black wing spot.

Female. Length 12 mm. *Head:* sides of face and narrow margin of front yellow. The facial tubercle, median stripe, vertex, and front except for a yellowish pre-antennal spot in the middle of which is a black spot, are all black. Antennae black, the base of third joint below

brownish. *Thorax:* metallic, brassy, brownish-black, the humeri and notapleurae are somewhat lighter brown and in the middle of the mesonotum, there are a pair of widely separated obscure pollinose vittae. Scutellum light brown, white pubescent, very short, sparse, black pilose, the ventral fringe is yellowish. *Abdomen:* slightly longer than wings, quite spatulate and chiefly dark brownish-black with pale pattern as follows: the anterior corners of third-fourth and fifth segments and also, narrowly separated, diffuse, elongate, rather small, vittate, paired spots in the middle of each of these segments. Those of the fifth segment are connected by way of the basal margin with the anterior corners. Sixth segment not quite as long as wide. Legs chiefly blackish, the anterior pair lighter, basal half of first and second tibiae whitish. Apical third of hind metatarsi and remaining joints whitish. *Wings:* broadly black on the anterior half of the basal third, widely and completely across the middle, and the whole of the subcostal cell and a conspicuous apical spot blackish.

Holotype: one female. Dec., 1940, J. Lane, collector.

Mesogramma lanei n. sp.

Related to *M. planiventris* Lw. but with narrower abdomen, pattern differences, femoral annulae and other differences.

Female. Length 4 mm. *Head:* face yellow, the tubercle brown tipped, vertex and the front broadly metallic black, its sides narrowly yellow. Antennae orange, brownish above. *Thorax:* mesonotum conspicuously brassy brown pollinose with a median steel blue vittae, the lateral margin widely tinged with yellow, the propleurae, humeri pale yellow, the scutellum brownish yellow with long sparse black pile. *Abdomen:* oval, yellowish brown marked with black as follows: posterior half of first segment, a narrow basal fascia and a slightly wider posterior fascia on the second segment. Third and fourth segment each with a posterior mar; ginal fascia that is somewhat less in width than one-fourth of the segments length. In the middles of the light yellow-brown areas left on these segments there are a pair of narrowly separated comma-like spots lying not very far from the anterior margin of the segments; their posterior corners form attenuated, slender, linear vittae that connect with the posterior fascia. Fourth segment with a medial, basal blackish spot and a lateral marginal fascia on the posterior half of the segment and the posterior margin of the segment in the middle, all black. *Legs:* pale yellow, a subapical blackish annulus on the hind femora and a faint similar subbasal annulus on the hind tibiae. Hind meta tarsi blackish brown and their last three joints similar. *Wings:* hyaline; stigma pale yellow.

Holotype: female, Tinga, Rio De Janeiro, Brazil, May 1940. Paratype, one female, same data in the collection of J. Lane.

Effective Living

This textbook for senior high school students was designed to help them discover ways of effective living. To live effectively they must consider themselves as individuals, as members of a family, and as useful citizens of a community; these three aspects form the organization of the book. Its content, however, is limited mainly to health knowledge and practises; so it is really a book of individual, family, and community hygiene. Although it is authoritative in its method of presentation (like most textbooks!), the information is interesting and the health practises sound. An excellent appendix is included on the control of communicable diseases.

—*J. G. Haub.*

Effective Living, by C. E. Turner and Elizabeth McHose, 432 pp., with 164 illustrations St. Louis, The C. V. Mosby Co., 1941.

GASTROINTESTINAL INTUBATION OF THE INTACT UNANESTHETIZED DOG[1]

R. E. MacQUIGG, M.A., M.D.
AND
F. J. KINGMA, D.V.M., M.Sc.

Intubation of the dog, for both motor and biochemical studies, has long been a fundamental part of classic physiology because of the availability of the dog and the general resemblance of its function to that of the human.

Fistulae and pouches of many kinds have been devised for all levels of the gastrointestinal tract; these, however, require some surgical facility and aftercare. Moreover, they are open to the objection of an altered physiology as well as

Fig. 1. Barium coated balloon on Rehfuss tube, in stomach of dog.

anatomy, which can often be disproved for a given purpose only by comparison with intubation of the intact animal. This usually requires training, which is troublesome and occasionally open to objection because of conditioned reflexes created, or it involves restraints to prevent the animal from displacing or even biting through and swallowing the tube. If the animal is made markedly uncomfortable, there are derangements throughout the organism as demonstrated for the stomach as early as 1916. (1) This is unfortunate particularly because of the promising possibilities in quantitative studies over periods of several hours.

[1]From the Departments of Research Surgery (G. M. Curtis, Chairman) and Veterinary Physiology and Pharmacology (D. W. Ashcraft, Chairman), Ohio State University. This investigation was aided by a grant from the Comly Fund of Ohio State University.

New fields have been opened by the development of the Miller-Abbott tube in its various forms. (2) These utilize the principle of propulsion of a multiple lumen tube by peristalsis acting on a balloon inflated after it has passed the pyloric sphincter. This leaves the other lumina free for aspiration or for registration of contractions with similar balloons. Barium may be injected through the tube to outline the gut, and the tube itself is sufficiently opaque to permit X-ray observation of its course. Figure 1 is a plate of the tube used for routine gastric motility work—a Rehfuss gastro-duodenal tube with the bucket replaced by a balloon (distal third of standard condom). A centimeter length of small glass tubing is slipped into the tip of the tube to prevent occlusion of its lumen by the silk thread used to tie the balloon. A pinch of barium sulfate mixed with a lubricating jelly free from grease (e. g., a tragacanth base) is placed in the balloon where it distributes itself and remains indefinitely without clogging. Such a tube does not

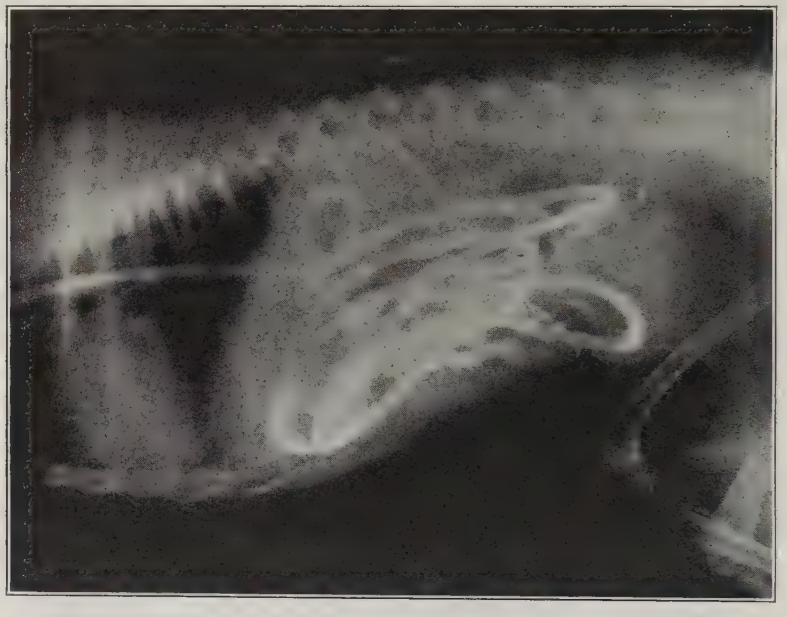

Fig. 2. Miller-Abbott tube at four-foot level (ileum) of dog.

permit aspiration or tandem balloon studies, for which the Miller-Abbott tube is used. Figure 2 shows the latter passed to the four foot level in a dog (with a small barium injection).

Refinements of technique (such as isolation of a section of gut by simultaneous inflation of a pair of balloons forming seals of proved efficiency) (3) now are appearing to make possible in the intact individual procedures formerly requiring surgery.

Following this trend toward more physiological, more chronic and more quantitative experimentation upon the digestive tract, we have developed a technique for intubation of the intact dog without training. One occasionally encounters an animal whose psychology precludes any co-operation, and which is so disturbed at being forced to remain quiet with an apparatus that the results are unsatisfactory for many purposes, and it may be that such animals constitute an irreducible minimum of unsuitable subjects. Usually, upon finding that struggle is useless and that relaxation brings comfort, the subject remains quiet.

The essence of the method is to place a bit made of transparent bakelite[2] in the dog's mouth, with the cross piece against the rear of the canine teeth. Here a natural space exists because of the rudimentary nature of the teeth immediately posterior to the canines. The bit is held against the roof of the mouth by a rubber strap over the upper jaw. (See Fig. 3.) The tube is threaded through this bit, which extends sufficiently to the rear to minimize coiling in the mouth and to prevent engagement of the tube by the molars.

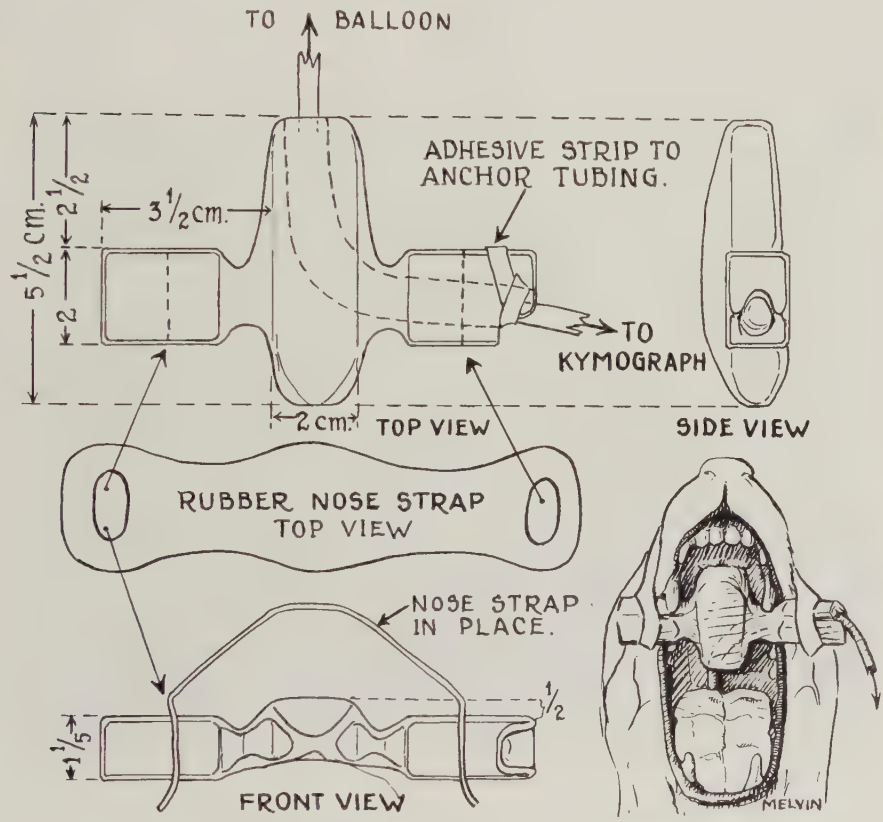

Fig. 3. Details of bit used as tube retainer.

When gastric intubation is sufficient, the tunnel for the tube is carried out through an end of the cross piece, which is cut away to provide a shoulder against which the tube may be taped to maintain it at the proper level. In intestinal work, where it is desirable to have the tube feed with the utmost freedom, (so that peristalsis will be sufficient to advance it at the proper rate) (2) the hole in the bit may be drilled straight through the body.

The bakelite is readily sawed and drilled with the common woodworking tools. It may be shaped with a half-round rasp and a round file. The curved tunnel for the tubing, after being drilled from each end, may be rounded out by threading a cord of hemp or twisted manila paper through the passage, fastening the free end to a clamp, and sawing the bit to and fro. The bit is filed to clear the canine and

[2]Obtainable from the Bakelite Corp., Park Avenue, New York City.

second premolar, and grooved or pitted slightly to place no weight on the first premolar. The teeth then act merely as guides to limit anterior and lateral displacement, the strap keeping the bit forward because of the slope of the nasal bones. The strap (cut from a discarded inner tube) is necked near each end to allow the edges to roll away from the nose. The whiskers may be trimmed.

A bit of the size given will fit any dog of the terrier type and weighing between twenty and thirty-five pounds. The jaws rest upon the bit without discomfort, and the animal can pant or vomit without hindrance from bit or tube. Surprisingly, gag reflex is rarely evoked by the tube. Mineral oil is suggested for lubricant because it is physiologically inert, though the tubing should be cleaned after use because of the possible deteriorating effect of oil on rubber.

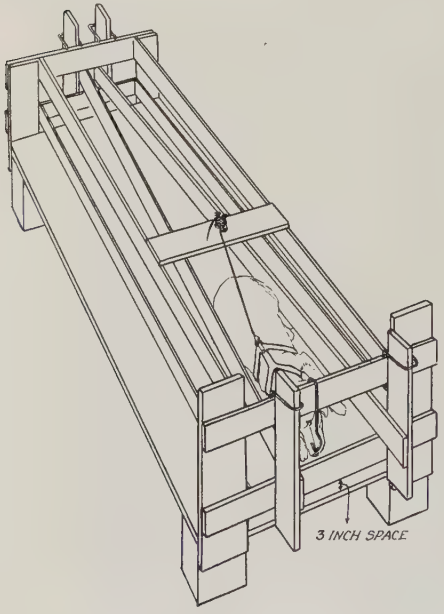

Fig. 4.
Adjustable restraining table.

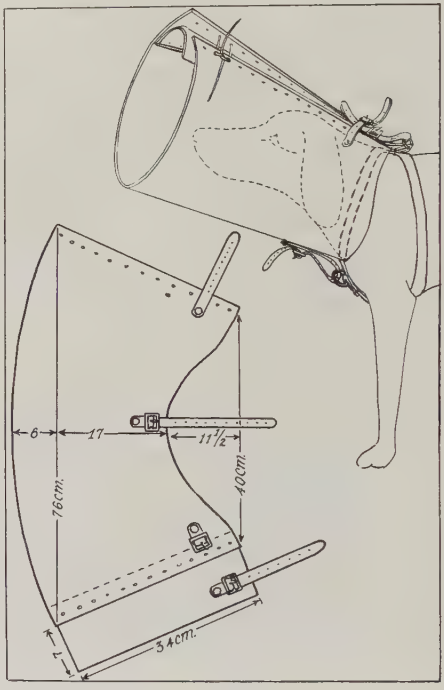

Fig. 5.
Modified Holmes funnel collar.

The dogs are kept in place by an adjustable stall and a standard shoulder harness, thus allowing them to lie upon either side or to stand, but not to leave the table nor to turn end for end and tangle the tube. (Fig. 4.) Dogs which insist upon pawing at the bit and tube are fitted with a modification of the Holmes funnel collar (4) which is strapped to the shoulder harness. An extra loop is riveted to the breastpiece of the harness for this purpose. (Fig. 5.) The shoulder harness should be of stout leather and well fitted, lest small loose-skinned animals learn to wriggle out of it, and to get their heads out of the funnel collar. Most animals, having tested to their satisfaction their inability to dislodge this apparatus, will lie quietly during protracted experiments, although occasional stroking by an attendant will reduce restlessness.

The region over the forearm is cleared with a depilatory, and intravenous injections may be given with a sharp 25 gauge needle into the radial vein. If an

attendant has plucked at the animal elsewhere to distract it, injections are unrecognized and hence made without evidence of psychic disturbance, as the animal lies hooded in the restraining frame. The side bars of the latter clear the table top by three inches, so that the legs of the animal may be drawn out as required. A metal table top is readily cleaned, affords the animals no traction, and they find it cool. It will be noted that the middle bar on each side of the frame may be moved in or out to accommodate varying sizes of animals and to vary the degree of restriction. The frame may be converted into a coop to further discourage movement by hinging a lattice to the bar upon which the leash is fastened, thus completely enclosing the animal.

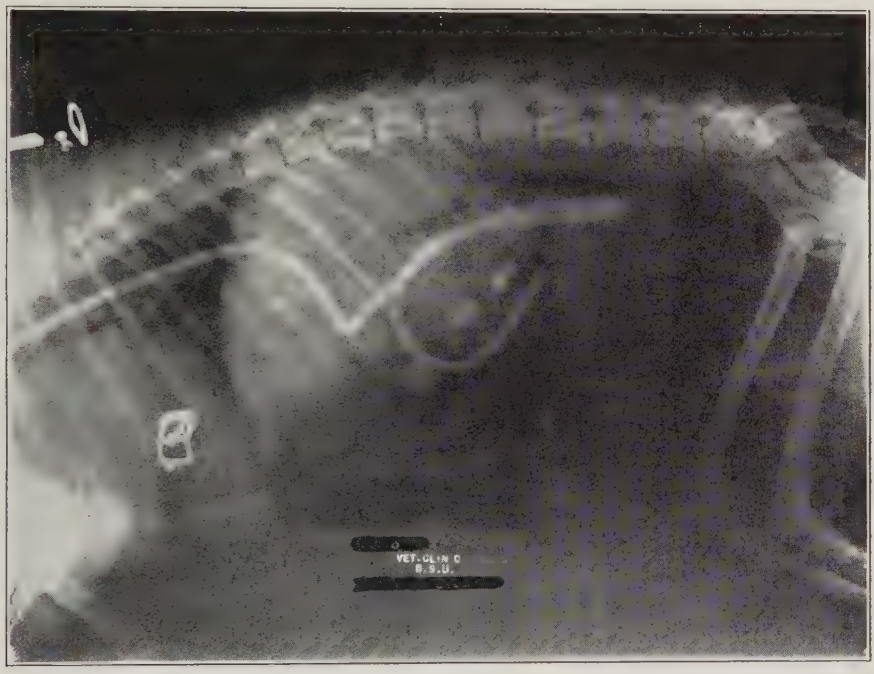

Fig. 6. Miller-Abbott tube being pulled through pylorus by pilot balloons.

The above methods have proved adequate for gastric intubation in all cases. Only in certain dogs will the Miller-Abbott tube readily pass the pyloric sphincter. For intestinal work one has the choice of testing until such an animal is found or performing a pyloroplasty. In the first case the tube should be fed very slowly, or taped to the bit at a level allowing several inches for duodenal penetration, and the balloon periodically inflated to see if it has dropped past the sphincter. If operation is used, the animal is no longer strictly intact, but if a Rammstedt, or even a modified Horsley operation is done, no fistula exists and there is negligible interference with the nerve and blood supply of the region and no undesirable fixation of the gut wall. In any case the level attained is identified by kymographic observation of the contractions upon the pilot balloon (duodenal motility is markedly different from that of the stomach) as well as by examining the fluids aspirated through the free lumen. (5)

Attempts have been made to devise a more positive method for passing the pyloric sphincter. A powerful magnet, acting on a ferrous bucket placed on the

tube, was tried and it was found at open operation that factors such as the spasticity of the sphincter and the presence of the rugae and of material in the stomach far outweighed the influence of the magnetic field at the distance through which the magnet must act. It was observed that coiling in the stomach was a prime obstacle, since it meant that the tip of the tube was in an unfavorable direction much of the time. (6) Since stiffer tubes seem to eliminate this, but are not sufficiently flexible for free passage to all levels, attempts were made to stiffen the tube until after passage of the sphincter by use of a wire stylet in the tube or of an external sleeve. Despite the aid of the fluoroscope, such methods have to date been disappointing. Most promising has been use of a tiny free pilot balloon on the end of six inches of string, which under the influence of duodenal peristalsis will bring the tip of the tube into position for passage of the pylorus during a moment of relaxation. If this pilot balloon contains barium, periodic fluoroscopic observation discloses the proper time for passing additional tubing to facilitate this, once the guide string has been engaged and can prevent the tube tip from recurving into the stomach and coiling. (Fig. 6) Further work is being done on this subject, which is of prime importance not only in facilitating research, but to the doctors throughout the country faced with urgent intubation of the seriously ill. Such methods as use of amyl nitrite to relax the sphincter (incidentally inhibiting peristalsis and causing circulatory disturbance) or of mercury to weight the pilot balloon, have obvious contra-indications or disadvantages.

SUMMARY

Methods are described for gastrointestinal intubation of intact, untrained and unanesthetized dogs, utilizing a bakelite bit which is strapped to the roof of the mouth, which has an extension carrying the tube clear of the molars and a cross piece fitted behind the canine teeth. The animals are placed in a stall allowing free motion up to the point of interference with the tube. A funnel collar is provided for unco-operative animals. Application of the Miller-Abbott principle of intestinal intubation to the dog is described, and the optimal method for passage of the pyloric sphincter is discussed.

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The Advancing Front of Medicine

In *Advancing Front of Medicine* Mr. Gray has written for the layman another book on science. His ability to give scientific information in clear, pleasing and understandable English without the usual exaggerations and misinterpretations is remarkable, especially for one trained as a reporter. His new book is interesting and easy reading. It is a book to have on the library table or at the bedside (if one must read in bed) where a chapter or so may be done as time permits. Some of the chapters of wide appeal are "Smoke," "Drink," "Cancer," "Allergies," "Sleep," and "Aging." His brief interesting account of the development of the "sulfa" compounds should be read by all laymen.

More writers of Gray's type are needed to bring the findings of research and of professional practice into the scope of the public understanding.

The Advancing Front of Medicine, by George W. Gray; buckram, 425 pp. Whittlesey House of The McGraw-Hill Book Company, New York, 1941. $3.00.—*D. F. Miller.*

NEW RECORDS OF OHIO DRAGONFLIES (ODONATA)

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and

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Up to the present time, 131 species of Odonata have been recorded for Ohio. The latest annotated list (Borror, 1937) listed 127 species; *Macromia pacifica* Hagen and *Enallagma basidens* Calvert were added by Borror in 1938; *Argia fumipennis* (Burm.) was added in 1939 by Wright (a specimen taken in a Franklin County greenhouse in January); and a record by Langlois (1932) of *Coryphaeschna* sp. (probably *ingens* Rambur), was overlooked in the later lists.

Recently a number of records have been obtained which add another species to the state list and a number of new county records, and which extend the extreme dates of a number of species' occurrence in the state.

The new species for the state list is as follows:

132. *Dorocordulia libera* (Selys). 1♂ and 1♀, June 22, 1924, by John C. Pallister. The specimens were taken separately, flying near an open woods, at Bradley Pond in Geauga County. These specimens are No. 32590 and No. 32591 in the collection of the Cleveland Museum of Natural History. This species occurs in northeastern United States and Canada, around the boggy shores of ponds. It has been taken in northern Indiana and in Michigan, and probably occurs at other ponds in northern Ohio.

The new county records obtained from material recently examined by the writers are listed below. In this list the name of collectors (when known) are indicated by abbreviations; the location of specimens, when in another collection than that of the collector, is indicated by abbreviations in parentheses. The following abbreviations are used for collectors and collections:

CHK.....Clarence H. Kennedy
CMNH...Cleveland Museum of Natural History
DJB.....Donald J. Borror
EE.....Erwin J. Epstein
EST.....Edward S. Thomas
GL.....G. Lucas
HMS.....H. M. Spandau
JD.....Julian L. Dusi
JP.....John C. Pallister
JRP.....John R. Patton
JST.....John S. Thomas
MT.....Marie Todd
MW.....Mike Wright
OG.....Orville Gundrum
OSM.....Ohio State Natural History Museum
OSU.....Dept. Entomology, Ohio State University
RG.....Robert Goslin
WLH.....William L. Hughes
WRP.....William R. Piper

The new county records are as follows (the species are numbered as in Borror's 1937 annotated list):

1. *Tachopteryx thoreyi* (Hagen). Cuyahoga, EE (CMNH).
4. *Ophiogomphus rupinsulensis* (Walsh). Ashland, CHK; Delaware, CHK; Geauga, JP (CMNH); Medina, JP (CMNH).
10. *Gomphus fraternus* (Say). Ashland, CHK; Muskingum, (DJB).
13. *Gomphus lineatifrons* Calvert. Delaware, MW.

14. *Gomphus lividus* Selys. Ashland, CHK; Delaware, DJB; Ashtabula, JP (CMNH).
18. *Gomphus spicatus* Hagen. Cuyahoga, JP (CMNH).
20. *Gomphus vastus* Walsh. Ashland, CHK.
27. *Gomphaeschna antilope* (Hagen).[1] Fairfield, RG (OSM).
28. *Basiaeschna janata* (Say). Summit, JP (CMNH).
30. *Boyeria vinosa* (Say). Licking, EST (OSM).
31. *Anax junius* (Drury). Cuyahoga, EE (CMNH); Lake, EE (CMNH); Summit, JP (CMNH).
32. *Anax longipes* Hagen. Ottawa, WLH (OSU).
39. *Aeshna verticalis* Hagen. Cuyahoga, JP (CMNH).
40. *Cordulegaster diastatops* (Selys). Medina, JP (CMNH).
42. *Cordulegaster maculatus* Selys. Portage, JP (CMNH).
43. *Cordulegaster obliquus* (Say). Adams, JRP (MW).
44. *Macromia illinoiensis* Walsh. Ashland (seen by CHK).
50. *Tetragoneuria cynosura* (Say). Lake, EE (CMNH); Summit, MT (CMNH).
54. *Perithemis tenera* (Say). Cuyahoga, EE (CMNH), Geauga, JP (CMNH).
55. *Celithemis elisa* (Hagen). Lake, EE (CMNH); Richland, HMS.
56. *Celithemis eponina* (Drury). Lake, EE (CMNH).
62. *Libellula cyanea* Fabricius. Highland, GL (DJB).
64. *Libellula luctuosa* Burmeister. Cuyahoga, EE (CMNH); Lake, EE (CMNH); Summit, JP (CMNH).
65. *Plathemis lydia* (Drury). Cuyahoga, EE (CMNH); Lake, EE (CMNH).
66. *Libellula pulchella* Drury. Cuyahoga, EE (CMNH); Lake, EE (CMNH); Lorain, OG (CMNH); Medina, JP (CMNH).
67. *Libellula quadrimaculata* Linnaeus. Portage, JP (CMNH).
68. *Libellula semifasciata* Burmeister. Cuyahoga, JP (CMNH).
70. *Sympetrum ambiguum* (Rambur). Greene, JST (OSM).
72. *Sympetrum obtrusum* (Hagen). Lake, EE (CMNH).
73. *Sympetrum rubicundulum* (Say). Cuyahoga, EE (CMNH); Lake, EE (CMNH); Lorain, OG (CMNH); Richland, HMS.
74. *Sympetrum semicinctum* (Say). Cuyahoga, JP (CMNH); Geauga, JP (CMNH).
76. *Sympetrum vicinum* (Hagen). Adams, MW; Cuyahoga, EE (CMNH); Lake, EE (CMNH).
77. *Leucorrhinia intacta* Hagen. Cuyahoga, EE (CMNH); Lake, EE (CMNH).
78. *Pachydiplax longipennis* (Burmeister). Cuyahoga, EE (CMNH).
79. *Erythemis simplicicollis* (Say). Cuyahoga, JP (CMNH); Lake, EE (CMNH); Summit, JP (CMNH).
80. *Pantala flavescens* (Fabricius). Cuyahoga, JP (CMNH).
87. *Agrion maculatum* Beauvais. Cuyahoga, EE (CMNH); Lorain, OG (CMNH).
88. *Hetaerina americana* (Fabricius). Cuyahoga, JP (CMNH); Morrow, JD.
91. *Lestes congener* Hagen. Cuyahoga, EE (CMNH); Lake, EE (CMNH).
92. *Lestes disjunctus* Selys. Hocking, DJB.
94. *Lestes forcipatus* Rambur. Lake, EE (CMNH).
95. *Lestes inaequalis* Walsh. Cuyahoga, EE (CMNH).
96. *Lestes rectangularis* Say. Cuyahoga, EE (CMNH); Lake, EE (CMNH).
98. *Lestes unguiculatus* Hagen. Cuyahoga, EE (CMNH); Lake, EE (CMNH).
100. *Argia apicalis* (Say). Cuyahoga, EE (CMNH).
102. *Argia moesta* (Hagen). Cuyahoga, JP (CMNH).
105. *Argia violacea* (Hagen). Cuyahoga, EE (CMNH).
106. *Amphiagrion saucium* (Burmeister). Cuyahoga, JP (CMNH); Medina, JP (CMNH).
108. *Nehalennia irene* Hagen. Cuyahoga, EE (CMNH).
109. *Chromagrion conditum* (Hagen). Cuyahoga, EE (CMNH); Summit, JP (CMNH).
110. *Enallagma antennatum* (Say). Cuyahoga, EE (CMNH); Lake, EE (CMNH).
114. *Enallagma civile* (Hagen). Cuyahoga, EE (CMNH); Lake, EE (CMNH); Richland, HMS.
117. *Enallagma ebrium* (Hagen). Lake, EE (CMNH).
118. *Enallagma exsulans* (Hagen). Shelby, WRP.
120. *Enallagma hageni* (Walsh). Medina, JP (CMNH).
121. *Enallagma signatum* (Hagen). Cuyahoga, EE (CMNH).
126. *Ischnura verticalis* (Say). Cuyahoga, EE (CMNH).
127. *Anomalagrion hastatum* (Say). Lucas, EST (OSM).

Many of the recently obtained records of Ohio Odonata have necessitated a revision of the extreme dates for a number of species' occurrence in Ohio; these revised extreme dates are as follows:

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[1]Gloyd (1940) has shown that the specimen recorded as *G. furcillata* (Say) by Borror (1937) is *G. antilope* (Hagen), a species distinct from *furcillata* (Say).

3. *Hagenius brevistylus* Selys. May 30 to Aug. 4.
4. *Ophiogomphus rupinsulensis* (Walsh). May 17 to July 28.
6. *Gomphus abbreviatus* Hagen. June 10 to June 14.[2]
16. *Gomphus plagiatus* Selys. May 25 to July 1.
20. *Gomphus vastus* Walsh. May 30 to July 15.[3]
23. *Gomphus viridifrons* Hine. June 7 to June 14.
27. *Gomphaeschna antilope* (Hagen). June 15 to June 19.
31. *Anax junius* (Drury). April 4 to October 25.
37. *Aeshna mutata* Hagen. May 24 to June 27.
39. *Aeshna verticalis* Hagen. August 26 to October 6.
40. *Cordulegaster diastatops* (Selys). May 21 to July 10.
50. *Tetragoneuria cynosura* (Say). May 8 to August 10.
54. *Perithemis tenera* (Say). June 13 to September 20.
62. *Libellula cyanea* Fabricius. June 6 to September 14.
65. *Plathemis lydia* (Drury). May 13 to September 9.
67. *Libellula quadrimaculata* Linneaus. May 15 to June 22.
72. *Sympetrum obtrusum* (Hagen). June 23 to September 18.
73. *Sympetrum rubicundulum* (Say). May 30 to September 18.
74. *Sympetrum semicinctum* (Say). July 2 to September 21.
79. *Erythemis simplicicollis* (Say). May 19 to October 18.
94. *Lestes forcipatus* Rambur. April 27 to August 18.
95. *Lestes inaequalis* Walsh. June 13 to August 10.
96. *Lestes rectangularis* Say. May 30 to August 26.
98. *Lestes unguiculatus* Hagen. June 12 to August 28.
99. *Lestes vigalix* Hagen. June 19 to August 1.
102. *Argia moesta* (Hagen). May 26 to August 29.
103. *Argia sedula* (Hagen). June 12 to August 30.
104. *Argia tibialis* (Rambur). May 30 to August 2.
110. *Enallagma antennatum* (Say). May 21 to August 30.
120. *Enallagma hageni* (Walsh). May 24 to July 10.

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[2]An earlier paper (Borror, 1937) gives an inaccurate extreme date.
[3]An earlier paper (Borror, 1937) gives an inaccurate extreme date.

A New Angle on Infectious Disease

This is a work of much interest to the biologist or to anyone with some biological training. It was written by a medical man but from the biological point of view instead of that of pure medicine. It is authoritative but not too technical. As is indicated from the title the departure is quite intentional. Infectious diseases are understood to be of bacterial, virus and protozoan origins. The approach made by the author is that disease is an ecological problem and may be so studied. Consideration is given to natural defenses, acquired immunities, epidemics, and principles of control and these make up a major portion of the book. A special chapter is devoted to diphtheria, tuberculosis, influenza, plague, cholera, malaria, and yellow fever. The final chapter on "New Diseases and the Outlook for the Future" was written before the outbreak of the present war. Dr. Burnet, the author, is Assistant Director of the Walter and Ellen Hall Institute in Melbourne, Australia.

Biological Aspects of Infectious Disease, by F. M. Burnet, M. D.; 310 pp. Cambridge: at The University Press; New York: The Macmillan Co., 1940. $3.75.—*D. F. Miller.*

A SYSTEMATIC STUDY OF THE MAIN ARTERIES IN THE REGION OF THE HEART—AVES—III.

THE FRINGILLIDAE. Part I

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INTRODUCTION

Recently the writer has discussed the arrangement of the arteries in the region of the heart of birds (3, 5, 6), the presence of a functional left radix aorta in the Kingfisher (2), and the presence of the ligamentum botalli in three species of birds (4).

In an earlier paper (3), the writer has pointed out that, according to Beddard (1), the left carotid artery alone persists in the Passeres—now Passeriformes. It has been this writer's experience that this is not the condition in the American passerine birds. Studies carried out on 30 species of the Fringillidae have resulted in the observations which are set forth in this paper.

MATERIALS

Routine dissections and observations were made on 30 species of the family Fringillidae in the preparation of this paper. Specimens were obtained from the United States National Museum, the Cleveland Museum of Natural History, and the Summit County Wildlife Research Station, Akron.

Specimens of the following species of birds were selected for study: *Carduelis carduelis, Certhidea cinerascens, Coryphospingus pileatus, Emberiza aureola, Emberiza flaviventris, Fringilla coelebs, Fringilla montifringilla, Geospiza f. fuliginosa, Geospiza s. septentrionalis, Guiraca caerulea, Hedymeles ludovicianus, Junco h. hyemalis, Leucosticte griseonucha, Loxigilla violacea affinis, Loxigilla noctis sclateri, Loxipasser anoxantha, Melospiza m. melodia, Melopyrrha nigra, Paroaria capitata, Passerculus sandwichensis savanna, Pipilo e. erythrophthalmus, Pipilo fuscus mesoleucus, Pyrrhula pyrrhula, Richmondena c. cardinalis, Spizella m. monticola, Spizella p. passerina, Spizella p. pusilla, Sporophila aurita, Tiaris bicolor omissa,* and *Tiaris o. olivacea.*

Results of this study are set forth in the following observations:

OBSERVATIONS

With reference to the main arteries in the neck and thorax, the basic arrangement-pattern is the same as that in other passerine birds, and differs only in certain details. The greatest divergence within the family results from retention (as vestiges) of certain embryonic vessels. The more important of these vestiges are derived from (1) the left radix aorta, (2) the distal portion of the 6th aortic arch, and (3) the ductus caroticus. In all cases where the above mentioned vestiges are found to persist, they remain as ligamentous vestiges of the embryonic vessels and not as functional vessels in the adult bird.

Among the Fringillidae the following arrangement-pattern is basic. The innominate arteries arise from the aortic root and then pass forward and to the left and right sides of the median-longitudinal axis of the body. The right innominate artery gives rise to the right systemic arch which then continues posteriorly to form the dorsal aorta. The right innominate then gives rise to the right common

carotid artery and the subclavian artery. From its origin in the innominate artery, the common carotid passes anteriorly to the thyroid gland where it sends off a short thyroid artery, and then gives rise to several branch arteries: (1) the ventral external artery which supplies the trachea and other ventral neck tissues; (2) the lateral external artery which serves as an ascending oesophageal artery as well as the main supply for the lateral cervical musculature and lymphatic glands of the right side of the neck; (3) the vertebral artery which passes dorsally and then anteriorly in the region of the brachial plexus. The left innominate artery gives rise to the common carotid and subclavian arteries on the left side. The left carotid then passes anteriorly to the thyroid gland, sends off a short vessel to the thyroid, and then gives rise to two branches; the larger forms the ventral internal carotid which enters the hypapophysial canal; the other gives rise to the lateral superficial vertebral and the cervical carotid. Both left and right lateral cervical and the right ascending oesophageal are superficial vessels. The left lateral superficial supplies the tissues along the left side of the neck in much the same manner as does its complimentary right vessel. The subclavian arteries (left and right) give rise to (1) the coracoid major, (2) the internal mammary, (3) the axillary, and (4) two pectoral arteries (Fig. 1).

The above condition, without ligamentous vestiges of the embryonic vessels, is found in *Emberiza aureola* (291235)[1], *Geospiza f. fuliginosa* (224902), *Guiraca caerulea* (33978), *Melopyrrha nigra* (321134), and *Paroaria capitata* (227245). Slight modifications of this condition are found in the following 25 species which were studied.

Loxipasser anoxantha (41994) retained the ligamentum caroticum—the anterior insertion of which is located on the right common carotid artery (near the thyroid gland) and the posterior insertion on the right radix aorta, at its junction with the 4th right aortic arch (Fig. 2).

Loxigilla violacea affinis (225921) revealed the presence of a complete ligamentum caroticum, the ligamentous vestige of the left radix aorta, and (for a short distance along the right radix aorta) the distal portion of the right ligamentum botalli (Fig. 3).

In *Pipilo fuscus mesoleucus* (4310), the distal portion of the right ligamentum botalli persists, but the proximal portion is completely atrophied, and the left radix aorta possesses a lumen for a short distance. The anterior or distal portion of the left radix remains as a ligamentous vestige of the embryonic vessel (Fig. 4).

Richmondena c. cardinalis and *Spizella m. monticolla* are alike in arrangement. In both species the left radix aorta persists as a ligamentous vestige, and the ligamentum botalli is complete, attached to both the pulmonary artery and the right radix aorta (Fig. 5).

Leucosticte griseonucha (321135) lacks the right ligamentum botalli, but the left ligamentum botalli is present as is the ligamentous vestige of the left radix aorta and part of the presumed left 4th aortic arch (Fig. 6).

In *Junco h. hyemalis*, *Loxigilla noctis sclateri* (318215), *Melospiza m. melodia*, *Passerculus sandwichensis savanna*, *Pipilo e. erythrophthalmus*, *Spizella p. passerina*, and *Spizella p. pusilla* the left radix aorta remains as a fairly prominent ligamentous vestige; in *Carduelis carduelis* (226944), *Certhidea cinerascens* (321936), *Emberiza flaviventris* (227048), *Hedymeles ludovicianus*, and *Tiaris bicolor omissa* the left radix is reduced to a long thin very minute ligament; and in *Coryphospingus pileatus* (321953), *Pyrrhula pyrrhula* (292957), and *Sporophila aurita* (343928) the left radix remains as a short, though readily visible ligament. Still further reduction and modification of the left radix occurs in *Fringilla montifringilla* (291236) and *Tiaris o. olivacea* (291958), (Fig. 7). In the latter, it remains as a mere ligamentous button on the left side of the dorsal aorta, just anterior to the coeliac artery. In *Passerculus sandwichensis savanna* and *Pipilo e. erythrophthalmus*, the ductus shawi (5) is present and functional on both the left and right sides (Fig. 8).

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[1]The numbers after the scientific names indicate the specimen number for materials in the United States National Museum collection, Washington, D. C. Materials not numbered were obtained elsewhere.

In most of the species that were studied, the paired internal mammary arteries arise either as a branch of the coracoid major arteries, or from the subclavian arteries close to the base of the coracoid arteries. This relationship appears to be fairly constant in species of the family Fringillidae.

DISCUSSION

As the writer has previously stated (5), presence rather than absence of the ligamentous vestige of the left radix aorta is to be expected. In addition to the above structure, ligamentous vestiges of the ductus botalli, and occasionally the ductus caroticus or ligamentum caroticum, may be present in different species of birds.

It is the writer's opinion that degeneration of these embryonic vessels is a continuous process and may take place at different rates in different species of birds. This does not mean, however, that the ligamentous vestiges of these embryonic vessels will completely disappear during the adult life of the bird as already has been indicated, but that there is considerable post-embryonic atrophy of some of these embryonic vessels. Evidences for this will be presented in subsequent papers on the late-embryonic condition in the *Rhea*, and common chicken, and the arteries in the Black-billed cuckoo and 10-day-old chick.

It may be pointed out that the greatest similarity in the arrangement of the main arteries and the embryonic vestiges occur between the following species: *Spizella p. passerina* and *Spizella p. pusilla*, *Fringilla coelebs* and *Fringilla montifringilla*, and *Tiaris bicolor omissa* and *Tiaris o. olivacea*; while the greatest differences occur between *Geospiza f. fuliginosa* and *Geospiza s. septentrionalis*, *Emberiza aureola* and *Emberiza flaviventris*, *Loxigilla violacea affinis* and *Loxigilla noctis sclateri*, and *Pipilo fuscus mesoleucus* and *Pipilo e. erythrophthalmus*.

Presence of the ductus shawi in two species of passerine birds is not to be regarded as entirely anomalous, since the presence of this vessel was reported by the author in a previous paper (5). It is the writer's opinion that this vessel may

EXPLANATION OF FIGURES

Figures 1–8: Diagrammatic representations of the main arteries in the neck and thorax regions of the *Fringillidae*, ventral views.

1. Basic arrangement-pattern.
2. Loxipasser anoxantha.
3. Loxigilla violacea affinis.
4. Pipilo fuscus mesoleucus.
5. Richmondena c. cardinalis & Spizella m. monticolla.
6. Leucosticte griseonucha.
7. Commonest arrangement-pattern.
8. Passerculus sandwichensis savanna & Pipilo e. erythrophthalmus.

KEY TO ABBREVIATIONS

1. aortic root.
2. innominate artery.
3. pulmonary artery.
4. subclavian artery.
5. common carotid artery.
6. axillary artery.
7. pectoral arteries.
8. coracoid major artery.
9. internal mammary artery (intercostal artery).
10. left ventral internal artery.
11. left lateral superficial cervical.
12. right ascending oesophagus.
13. right lateral superficial cervical.
14. systemic (right 4th aortic) arch.
15. ligamentum caroticum.
16. ligamentous vestige of left radix aorta and ductus botalli.
17. right ligamentum botalli.
18. right vertebral artery.
19. left vertebral artery.
20. persistant left radix aorta (with lumen).
21. functional right radix aorta.
22. dorsal aorta.
23. ductus shawi (syrinx muscle arteries).

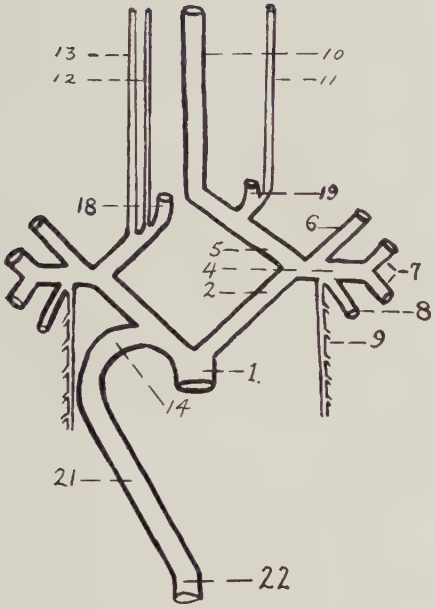

Figure 1

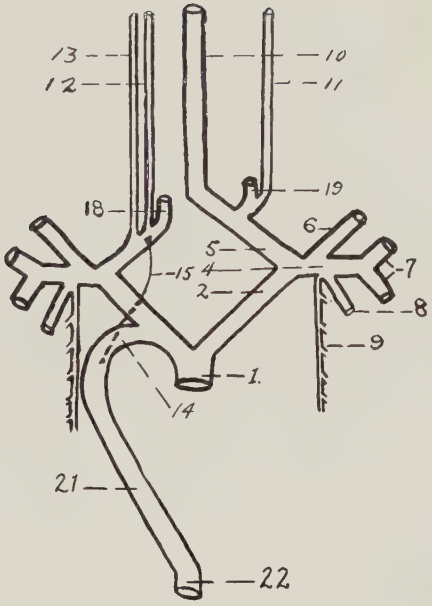

Figure 2

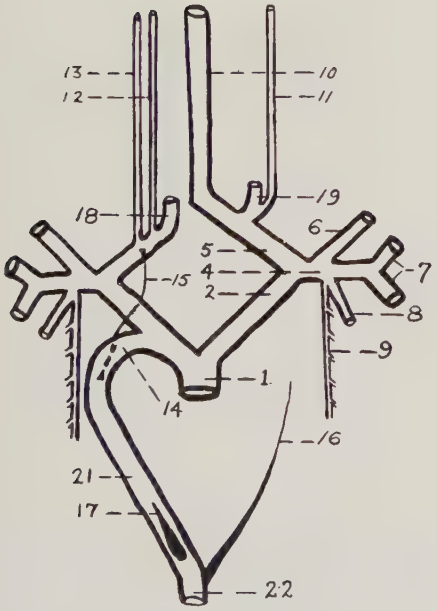

Figure 3

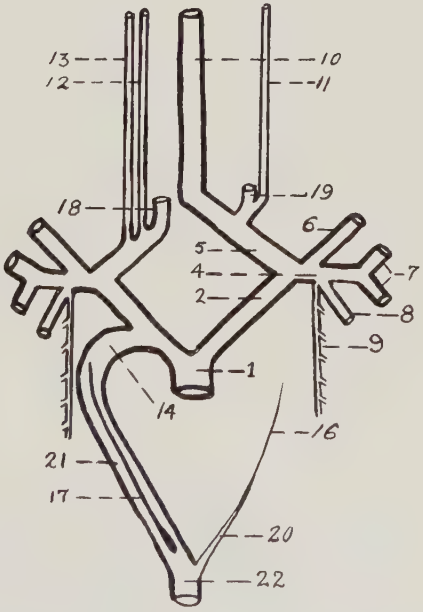

Figure 4

(See page 86 for explanation of figures and abbreviations)

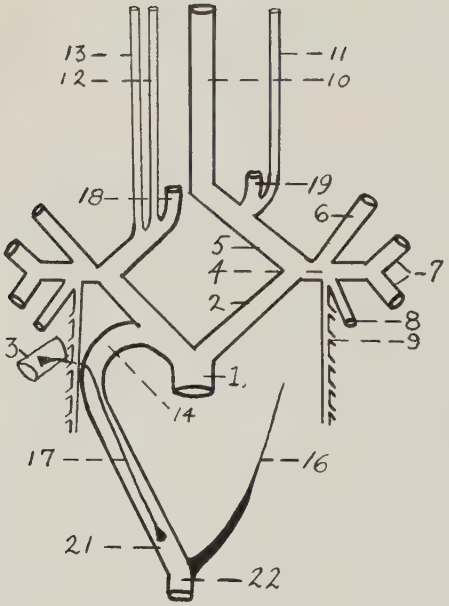

Figure 5

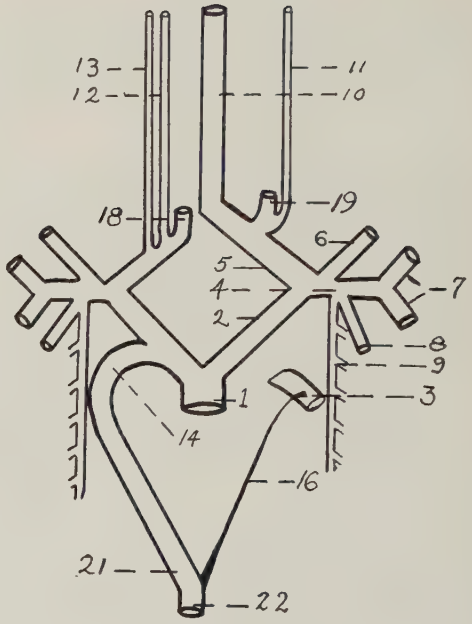

Figure 6

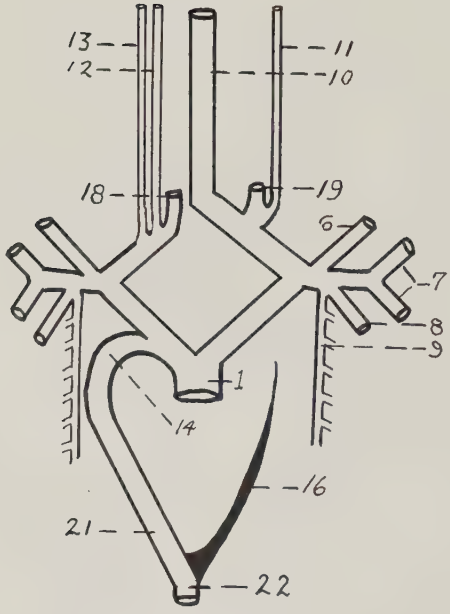

Figure 7

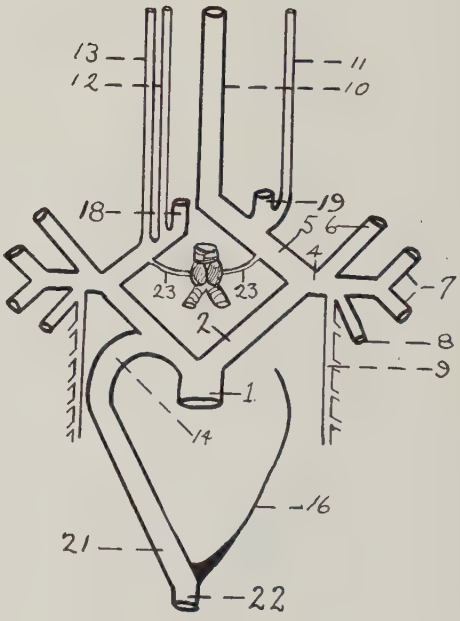

Figure 8

(See page 86 for explanation of figures and abbreviations)

be present in several other species of birds, but goes unrecognized due to its size along with difficulties encountered in ordinary methods of preservation and dissection. It may be that this short vessel is a vestige of the functional embryonic ductus caroticus, but evidence to support this has only recently been obtained.

It may be concluded that passerine birds possess a left common carotid artery, and that this vessel was not observed and recorded by the 19th century workers may have been due to faulty preparation and dissection, or to destruction of this vessel when the specimens were first taken.

In a recent paper (7), Wetmore indicated that it is possible that the Fringillidae have retained and carried more general characters from which further specialization may take place. He further points out that the Coerebidae and Icteridae—specialized families of the fringilline series—may have been derived as modifications from the Fringillidae. This is quite possible in the light of the present evidence, since the Fringillidae show a large number of variations in the arrangement of the arteries, with chief reference to retention of embryonic vestiges. Thus, it should be noted that in this study of 30 species of birds, eight modifications in pattern were observed. Thus far, the Icteridae which have been studied show but one pattern in arterial arrangement. It is likewise possible that slight variation in the basic pattern may be found in later studies, but such wide variations in pattern, as in the Fringillidae, is improbable.

For some time, the writer has been of the opinion that the Fringillidae do not represent the end of a line of evolution, and in this respect, the present studies tend to give increased evidences for Wetmore's contention that the Fringillidae represent the central group through which further progress (evolution) may be expected.

SUMMARY

1. Thirty species of the family Fringillidae were studied and the arrangements of the main arteries in the region of the heart, along with the ligamentous vestiges of certain embryonic vessels, were recorded.

2. The basic pattern for the functional vessels of the adult was found to be the same, but presence of certain embryonic vestiges varied in the different species.

3. The ductus shawi was found to be present and functional in two species.

4. In the majority of specimens, the left radix aorta was present and persisted as a ligament, while the ligamentum botalli persisted in fewer species.

5. The right common carotid artery was present, although earlier workers failed to report its presence, and considered it to be entirely lacking.

6. Although complete degeneration of certain embryonic vessels does not always occur in certain adult birds, it appears that there is some post-embryonic degeneration of these embryonic vessels.

7. The Fringillidae probably represent a central group through which further evolution may be expected.

ACKNOWLEDGMENTS

The writer wishes to express his gratitude to Dr. Alexander Wetmore and Dr. Herbert Friedmann, of the United States National Museum, and to Dr. John W. Aldrich, formerly of the Cleveland Museum of Natural History, for their co-operation and assistance in making a wide variety of specimens available for these studies; to his associates of the Summit County Wildlife Research Station for assistance in collecting and preparing specimens of local birds for study; and to Meems Bros. and Ward, of Oceanside, N. Y., for specimens so generously donated for this research; and finally to Dr. J. Paul Visscher, Dr. D. P. Quiring, and Dr. J. C. Gray, of the Department of Biology, Western Reserve University for their several suggestions during the course of these studies.

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Introduction to Biology

The text has been written for the "purpose of presenting southern plants and animals to students and teachers of southern high schools." Its subject matter is divided into eight parts: Introduction, The Simplest Animals, Plants without Seeds, The Seed-bearing Plants, Animals with Jointed Feet, Animals with Backbones, Structure and Function in Plants, and Structure and Function in Animals. There is a fairly complete glossary and an index at the end of the book. The 331 figures are mainly photographs and add much to the value of the book; some of the drawings, as the author admits, are amateurish.

The discussion is essentially a brief, not too technical, descriptive account of the plant and animal kingdoms. There is very little on physiological processes, even in the last two parts (though there is a good chapter on heredity), and there is either nothing at all or only incidental mention through the text of such subjects as ecology, conservation, and evolution. The experimental approach is not used, and there is little to arouse student thinking or to suggest student activities or projects. The book contains no bibliographic references or suggested supplementary readings.

The treatment of individual subjects, though of necessity brief in this sort of text, is in some cases so sketchy as to be misleading; this is particularly true in some of the chapters on insects. There are a few erroneous statements in the book: e. g., on p. 208 the copulatory apparatus of a male dragonfly is said to be on the fourth abdominal segment, when it is on the second; a photograph (fig. 244) of a museum skin of a swallow is labeled a horned lark skin.

As a high school text giving a brief survey of the plant and animal kingdoms, this book is probably satisfactory, but as an introduction to the general principles of biology it leaves much to be desired.—*D. J. Borror.*

An Introduction to Biology, by J. C. Cross, Ph.D. xviii+507 pp. St. Louis, The C. V. Mosby Company, 1941. $1.90.

Introducing Insects

This authoritative little book comes from the pen of one who is gifted in presenting to the layman the fundamentals of a usually regarded technical subject. With his own inimitable style he delves into some of our major insect problems and presents this information under the following headings: Why Study Insects; How to Study Insects; Butterflies; Dragonflies; Grasshoppers and Crickets; Leafbugs and Leafhoppers; Common Caterpillars; Common Leaf-eating Beetles; Scale Insects and Aphids; Mosquitoes; Insects that Eat Our Foods, Woolens, Fruits and Nuts; Carnivorous Insects; Bees, Indoors and Out; The Control of Insect Pests; How to Begin an Insect Collection; How to Rear Insects; Suggestions for Use of the Book. An index is included at the end of the book.

He uses only common names for the insects discussed and stresses the value of knowing their life history and habits in order to properly prescribe control measures. The illustrations which accompany the text should be of great aid to the user of the book, especially the beginner interested in entomology. The author is to be congratulated on the manner in which he is attempting to get scientific information on this subject before the public. Publications of this kind should be encouraged.—*R. H. Davidson.*

Introducing Insects, by James G. Needham. v+129 pp. Jaques Cattell Press, Lancaster, Penna., 1940. $1.50.

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BACTERIAL ACTIVITIES IN THE SUBAQUATIC SOILS OF LAKE ERIE

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During the summers of 1939 and 1940 a bacteriological study was made of the bottom soils of Lake Erie and its tributaries. This work was done for the Ohio Division of Conservation of which Dr. T. H. Langlois was director of research. Numerous samples were taken in different regions in order to study the role of micro-organisms in various physico-chemical phenomena. The area studied was divided into nine regions as follows: (1) Terwilliger's Pond which is a deep inlet directly connected with Hatchery Bay; (2) Hatchery Bay which connects Terwilliger's Pond with the lake proper; (3) Lake Erie proper which extends between the various islands; (4) Put-in-Bay Harbor which connects the lake proper with Squaw Harbor; (5) Squaw Harbor which is a shallow inlet of Put-in-Bay Harbor; (6) Pelee Island area extending around Pelee Island; (7) Portage River area; (8) Little Portage River area; (9) Haunck's Pond which is located in Middle Bass Island and is not directly connected with the lake unless wave action is quite great. All this region is in western Lake Erie within easy working distance from Put-in-Bay, Ohio. Particularly significant parts of the investigations dealt with the nitrogen transformations and the destruction of plant and animal materials, which have a direct bearing upon the quantity and quality of available food, and hence upon the fisheries industry.

METHODS AND TECHNICS

The samples were collected with dredges and placed in sterile wide-mouthed glass bottles. The samples were carefully removed from the center of the dredge core in order to reduce contamination to a minimum. Sterile wooden paddles were used in transferring samples to and from the bottles. Samples were tested immediately after being collected or were kept as cool as possible until examined. All technics employed were those recognized as standard in the bacteriological field. The agar plates were prepared by placing a gram of the soil in proper quantities of sterile distilled water to insure countable numbers of colonies on the agar plates. All inoculated media were incubated in electrically controlled incubators which operated at 20° C., 37° C. and 55° C. Agar plates were prepared in order to get an approximation of the relative numbers of organisms at various stations as revealed by the twenty-four and forty-eight hour incubation periods under aerobic and anaerobic conditions for each of the three temperatures mentioned (See Table 3).

In the study of the decomposition of mayflies a series of 500 cc. Erlenmeyer flasks was sterilized and inoculated with equal quantities of lake mud, lake water and adult mayflies as shown in Table 4. In samples AI, BI and CI the twenty mayflies were placed below the layer of mud and the flasks incubated at the tem-

peratures designated. In samples A2, B2 and C2 twenty similar mayflies were placed in each of the flasks but were allowed to float upon the surface of the water, and incubated at the temperatures designated. The results are shown in Table 4. The optimum temperature for the complete as well as the most rapid decomposition seems to be 37° C., or slightly below (as in the case of sample C1). When a temperature below 37° C. was satisfactory for mayfly decomposition the covering of the mayflies with a slight coat of mud seemed to expedite the process. However, when buried in the mud too deeply the mayflies became filled with gas and "popped" out of the mud before actual decomposition was started.

In the study of the decomposition of green algae a series of sterile test tubes was inoculated with similar quantities of algae, lake mud and lake water. In each tube the strand of algae was imbedded in the mud and the remainder of the tube filled with lake water. The results of the decomposition in the mud and in the water above the mud are shown in Table 5. As with the mayflies, the optimum temperature for complete decomposition of the algae was 37° C. although complete decomposition also took place in a much longer time at a lower temperature. A slight degree of decomposition also took place at 55° C. in 4 days but the amount of destruction never advanced beyond this point within 64 days when the experiments were concluded.

In order to measure the amounts of gas produced during the decomposition process, equal quantities of mayflies, green algae as well as *Ceratophyllum* (hornwort) were placed in different sterile half-gallon fruit jars. In each jar a measured quantity of lake mud was placed in the bottom of the jar. The mayflies, algae or *Ceratophyllum* was then placed upon the mud. An inverted funnel with an inverted test tube on its stem were then placed over the inoculum. The jar, funnel and test tube were then completely filled with lake water. Incubations were made at 37° C. and 20° C. Controls without plant or animal materials were incubated under the same conditions to eliminate the possibility of having the gas production come from the mud rather than the inoculum. The jars were lightly covered with paper to reduce contaminations. The gas produced by the decomposition of the materials under the funnels was collected in the inverted test tubes as the water was displaced from them. The results are shown in Table 6.

MEDIA AND TESTS

In addition to routine, standard media the following were used for specific purposes. The demonstration of organisms capable of reducing urea to ammonia was made in Kappen's urea broth which was alkalinized with ammonium carbonate. The test for ammonia was made with Nessler's reagent. The detection of organisms capable of oxidizing ammonia to nitrites was made in Winogradsky's ammonium sulphate medium. The test for nitrites was made with the sulphanilic acid-alpha naphthylamine reagent. The demonstration of organisms capable of oxidizing nitrites to nitrates was made in sodium nitrite medium. The test for nitrates was made with diphenylamine reagent. When the test for nitrite was negative, one cubic centimeter of the culture was placed in a clean sterile tube and 2 drops of diphenylamine reagent and 2 drops of concentrated sulphuric acid were added. A blue color indicated nitrates (if the nitrite test was negative). To destroy the nitrites which interfered with the test, the culture to which sulphanilic-acetic acid was added, was kept in a water bath (boiling) for 20 minutes. Tests were made after heating to determine if nitrites had been eliminated. Tests for nitrates were then made. The successive reduction of nitrates to nitrites, to ammonia, and to free nitrogen was demonstrated in nitrate broth in test tubes in which there was an inverted smaller tube to collect the gas. The formation of foam on the surface of the medium, or of gas in the inverted tube, or both, showed the presence of free nitrogen. The detection of organisms (*Azotobacter sp.*) capable

of fixing free nitrogen nonsymbiotically was done in Ashby's mannitol phosphate solution. The organisms appeared in a heavy, slimy pellicle after incubation under optimum conditions.

The test for cellulose-digesting organisms was made in McBeth's cellulose ammonium sulphate solution. Strips of sterile filter paper were placed in the tubed medium. After inoculation and incubation for the required period of time the following were noted: the time of paper discoloration; the evolution of gas; the odor; the time at which the paper began to decompose; when decomposition was complete (at which time Gram's stain was made). Eckford's method was used in determining the presence of organisms capable of hydrolyzing starch. Soluble starch (0.2%) was added to broth, and after proper incubation the medium was tested for acid production with an indicator of the proper pH-range. A drop of the inoculated medium was also placed in a depression of a porcelain plate and a drop of dilute iodine solution added. The reactions were read as follows: if blue, no hydrolysis; if reddish-brown, partial hydrolysis with the production of erythrodextrin; if clear, complete hydrolysis with the production of dextrin or perhaps glucose. Tests for sugars were made with Fehling's reagent. ZoBell and Feltham's technic for the determination of hydrogen sulphide was used. Ordinary broth was inoculated and incubated at the optimum temperature. A test strip of sterile filter paper soaked in a saturated solution of lead acetate was placed between the cotton plug and the mouth of the tube during incubation. Blackening of the test strip signified the presence of organisms capable of producing hydrogen sulphide. Indole-producing organisms were demonstrated by inoculating a solution of tryptone (0.1%). The Gnezda oxalic acid test showed the presence of indole. Strips of filter paper were immersed in a warm saturated solution of oxalic acid and then dried. A strip of the paper was inserted into the culture tube under aseptic conditions, and bent at such an angle that it pressed against the tube and remained near the mouth. The oxalic acid crystals on the paper turned pink if indole was formed. The presence of lactose-fermenting organisms was shown by inoculating lactose broth in Smith's fermentation tubes. The amount of gas was determined by means of a gasometer.

CONCLUSIONS

The data collected in connection with nitrogen transformations suggest the following conclusions:

(A) A reduction of urea to ammonia (in Kappen's urea broth) was observed in every sample in all stations tested. The reaction was recorded as strong in 42 stations (in 1½ to 3 days); moderate in 5 stations; and slight in 8 stations. It would appear that organisms capable of reducing urea to ammonia are quite generally distributed in the mud. (See Table 1).

(B) The oxidation of ammonia to nitrites (in Winogradsky's ammonium sulphate medium) was recorded as strong in 12 stations; moderate in 7 stations; slight in 27 stations; and negative in 9 stations. (See Table 1). These data would suggest that organisms capable of moderately or strongly oxidizing ammonia to nitrites, at least as revealed by laboratory conditions, are not as plentiful as the urea-reducing organisms.

(C) The oxidation of nitrites to nitrates (in sodium nitrite solution) was only slight in 15 stations and negative in 40 stations. (See Table 1). Even in stations where a slight test was positive, this occurred only after a minimum of 20 days. In many stations where a slight positive reaction occurred, a much longer time was required. It would appear that organisms capable of oxidizing nitrites to nitrates, at least as revealed by laboratory tests, are not as plentiful as the ammonia-oxidizing organisms. As a consequence, a minimum of nitrate formation for food purposes is to be expected. The real "bottle beck" in the production of nitrates

from urea seems to be the result of a minimum of nitrite-oxidizing organisms, at least as revealed by laboratory data.

TABLE 1

SUMMARY OF NITROGEN TRANSFORMATIONS

	Station Number and Location	Reduction of Urea to NH_3 (Kappen's Urea Broth)	Oxidation of NH_3 to NO_2 (Winogradsky's Ammonium sulphate Solution)	Oxidation of NO_2 to NO_3 (Sodium Nitrite Solution)	Reduction of NO_3 to NO_2, NH_3 and N_2 (Nitrate Broth)	Azotobacter Organisms (Present in Days)
Terwilliger's Pond	9	Strong (2 days)	Slight (11) days)	None (45 days)	Strong (2 days)	6 days
	10	" " "	None (60 days)	" 60 "	Moderate (2 days	7 "
	11	" " "	Slight (50 days)	" " "	" " "	6 "
	11x	" " "	None (35 days)	" 45 "	Strong (3 days)	8 "
	11a	" " "	" " "	" " "	" " "	7 "
	12	" " "	Moderate (45 days)	Slight (30 days)	Moderate (2 days)	7 "
	13	" " "	Slight (13 days)	" 22 "	Strong (2 days)	6 "
	14	" " "	None (60 days)	None (60 days)	" " "	4 "
	15	" " "	Moderate (40 days)	Slight (45 days)	" " "	5 "
Hatchery Bay	16	Strong (2 days)	Slight (35 days)	Slight (50 days)	Moderate (3 days)	5 days
	17	Moderate (3 days)	None (60 days)	None (60 days)	" " "	3 "
	18	Strong (3 days)	Slight (65 days)	Slight (50 days)	" " "	3 "
	19	" " "	None (60 days)	None (60 days)	" " "	4 "
Lake Erie	36	Slight (3 days)	Slight (8 days)	None (50 days)	Moderate (2 days)	5 days
	37	" " "	" " "	" " "	" " "	5 "
	38	" " "	" " "	" " "	" " "	5 "
	39	Strong (3 days)	Moderate (5 days)	" " "	Strong (2 days)	6 "
	40	" " "	Slight (4 days)	" " "	" " "	6 "
	41	" " "	Moderate (4 days)	Slight (35 days)	" " "	6 "
	42	" " "	Slight (4 days)	None (50 days)	" " "	6 "
	43	" " "	" " "	" " "	" " "	6 "
Put-in-Bay Harbor	20	Strong (3 days)	Strong (33 days)	Slight (40 days)	Strong (2 days)	6 days
	21	" " "	" " "	None (60 days)	Moderate (2 days)	6 "
	22	" " "	" 31 "	Slight 45 days)	" " "	6 "
	23	" " "	Moderate (30 days)	" 42 "	" " "	5 "
	24	" " "	Slight (40 days)	None (60 days)	" " "	8 "
	25	" " "	Moderate (40 days)	" " "	" " "	6 "
	26	" " "	Slight (19 days)	Slight (20 days)	" " "	5 "
Squaw Harbor	55	Strong (1½ days)	None (35 days)	None (35 days)	Strong (1 day	6 days
	56	" " "	" " "	" " "	" " "	5 "
	57	" 3 "	" " "	" " "	" " "	6 "
Pelee Island	88	Strong (1½ days)	Strong (2 days)	None (6 days)*	Strong (2 days)	7 days
	88a	" " "	" " "	" " " *	" " "	7 "
	89	" " "	" " "	" " " *	" " "	7 "
	89a	" " "	" " "	" " " *	" " "	7 "
Portage River	44	Very strong (2 days)	Slight (17 days)	None (45 days)	Strong (2 days)	3 days
	45	" " " "	" " "	" " "	" " "	5 "
	48	" " " "	Strong (17 days)	Slight (35 days)	Moderate (2 days)	3 "
	49	" " " "	Slight (17 days)	None (50 days)	" " "	3 "
	50	" " " "	" " "	" " "	" " "	3 "
	51	" " " "	" " "	" " "	" " "	3 "
	52	Moderate (2 days)	Strong (17 days)	Slight (30 days)	" " "	3 "
	53	Very strong (2 days)	" 15 "	None (50 days)	" " "	3 "
	54	" " " "	Moderate (16 days)	Slight (18 days)	Strong (2 days)	3 "
Little Portage River	46	Very strong (2 days)	Strong (17 days)	Slight (50 days)	Strong (2 days)	3 days
	47	" " " "	" " "	None (50 days)	" " "	3 "
Haunck's Pond	27	Strong (3 days)	Slight (35 days)	Slight (42 days)	Moderate (2 days)	6 days
	28	Moderate (3 days)	" 41 "	None (60 days)	" " "	6 "
	29	" " "	" 14 "	" " "	" " "	6 "
	30	" " "	" 40 "	" " "	" " "	6 "
	31	Slight (3 days)	" 14 "	" " "	" " "	6 "
	32	" " "	" 14 "	" " "	" " "	6 "
	33	" " "	" 16 "	" " "	" " "	6 "
	34	" " "	" 14 "	" " "	" " "	6 "
	35	" " "	" 18 "	" " "	" " "	6 "

* Experiments discontinued at end of period designated.

(D) The reduction of nitrates to nitrites, to ammonia, to free-nitrogen (in nitrate broth) showed a strong production of ammonia (1–2 days) in 24 stations and a moderate production in 31 stations. A strong test for free nitrogen was

present in 23 stations; moderate in 31 stations; and slight in 1 station. (See Table 1). These data suggest that organisms are prevalent which are capable of

TABLE 2

SUMMARY OF CELLULOSE DIGESTION, STARCH HYDROLYSIS, HYDROGEN SULPHIDE PRODUCTION, INDOLE PRODUCTION AND LACTOSE BROTH FERMENTATION

Station Number and Location		Cellulose Digestion	Starch Hydrolysis	Hydrogen Sulphide Production	Indole Production	Lactose Broth Percent of Gas	
						24 hours	48 hours
Terwilliger's Pond	9	None (30 days)	Complete (2 days)	Strong (1 day)	Strong (2 days)	60	90
	10	Complete (14 days)	" " "	" " "	Moderate (2 days)	35	70
	11	None (30 days)	" " "	Moderate (1 day)	Slight (2 days)	55	75
	11x	Complete (16 days)	Partial (35 days)*	Strong (2 days)	" 6 "	10	15
	11a	None (35 days)	" " " *	Slight (35 days)	" 8 "	5	10
	12	Complete (19 days)	Complete (2 days)	Strong (1 day)	Moderate (2 days)	70	95
	13	None (30 days)	" " "	" " "	Slight (2 days)	60	90
	14	Slight (21 days)	" " "	" " "	" " "	55	80
	15	" " "	" " "	" " "	" " "	50	70
Hatchery Bay	16	None (35 days)	Complete (3 days)	Moderate (3 days)	Moderate (4 days)	45	75
	17	" " "	" 4 "	" " "	" " "	45	55
	18	" " "	" " "	" " "	" " "	60	90
	19	" " "	" 3 "	" " "	" " "	55	65
Lake Erie	36	None (27 days)	Complete (2 days)	Strong (3 days)	Slight (10 days)	60	100
	37	Complete (16 days)	" " "	" " "	" " "	55	90
	38	None (26 days)	" " "	" " "	" " "	55	95
	39	" 30 "	" " "	" 2 "	" 3 "	55	80
	40	Complete (23 days)	" " " *	" " "	" 10 "	60	80
	41	None (30 days)	" " "	" " "	" 10 "	80	90
	42	Complete (25 days)	" " " *	" " "	None (10 days)	80	90
	43	" 20 "	" " " *	" " "	Strong (7 days)	70	85
Put-in-Bay Harbor	20	Complete (45 days)	Complete (3 days)	Slight (3 days)	Slight (4 days)	80	90
	21	" 16 "	" " "	None (10 days)	" " "	80	90
	22	" " "	" " " *	Strong (3 days)	" " "	60	80
	23	" 18 "	" " " *	None (3 days)	" " "	60	80
	24	None (48 days)	" " " *	" " "	" 6 "	65	80
	25	" " "	" " " *	Strong (3 days)	" 4 "	75	80
	26	Complete (16 days)	" " " *	Slight (3 days)	" 6 "	80	90
Squaw Harbor	55	Complete (14 days)	Partial (30 days)*	Slight (23 days)	Slight (6 days)	40	70
	56	" 16 "	" " "	" " "	" " "	50	75
	57	" 17 "	" 25 " *	Strong (3 days)	" 7 "	50	75
Pelee Island	88	Not tested	Partial (5 days)*	Slight (6 days)	None (6 days)	0	10
	88a	" "	" " " *	Moderate (5 days)	" " "	0	10
	89	" "	" " " *	" " "	" " "	0	10
	89a	" "	" " " *	" " "	" " "	0	10
Portage River	44	Complete (23 days)	Complete (2 days)	Strong (2 days)	None (6 days)	45	70
	45	" 14 "	" " "	" " "	" 7 "	60	90
	48	" 18 "	" " "	" " "	" " "	55	85
	49	None (25 days)	" " "	" " "	" " "	60	95
	50	Complete (25 days)	" " "	" " "	" " "	50	80
	51	" 26 "	" " "	Moderate (2 days)	" " "	45	70
	52	" 25 "	" " "	Strong (2 days)	Slight (4 days)	50	85
	53	" 22 "	" " "	" " "	" " "	55	90
	54	" 20 "	" " "	" " "	Strong (2 days)	65	95
Little Portage River	46	None (28 days)	Complete (2 days)	Strong (2 days)	None (7 days)	60	90
	47	Complete (25 days)	" " "	" " "	" " "	60	90
Haunck's Pond	27	Complete (13 days)	Complete (3 days)*	Strong (3 days)	Slight (6 days)	10	60
	28	None (50 days)	" " " *	" " "	" " "	15	70
	29	" " "	" 4 " *	" " "	" " "	50	65
	30	Complete (13 days)	" " " *	" " "	" " "	5	50
	31	" 19 "	" " " *	" " "	" " "	5	50
	32	" 14 "	" " " *	" " "	" " "	0	60
	33	" 10 "	" " " *	" " "	" " "	20	50
	34	" 12 "	" " " *	" " "	" " "	0	50
	35	" 17 "	" " " *	" " "	" " "	70	100

* Butyric acid odor.

reducing nitrates rather extensively. Consequently, the balance between nitrate formation and nitrate reduction appears to be in favor of the latter.

(E) The presence of nonsymbiotic, free-nitrogen-fixing *Azotobacter* organisms was demonstrated in each of the 55 stations in 3 to 7 days when incubated at 20° C. in Ashby's mannitol phosphate solution (100 cc. in a 500 cc. Erlenmeyer flask). (See Table 1). The presence of these organisms does not prove their ability to fix free nitrogen in such soils as are found in the lake. Specific investigations will have to be made in this direction to clear up this point.

A study of Table I reveals consistent results for all stations in certain regions while considerable variations were noted in the different stations of other regions. For instance, in the Terwilliger Pond region, certain stations showed no nitrite production within 60 days while other stations in the same region showed some nitrite formation within 11 days. Similar phenomena are to be observed in other

TABLE 3

Average for All Stations in Each Area	Incubation Temperature	Average Number of Colonies on Agar Plates (Per Gram of Soil)			
		Aerobic		Anaerobic	
		24 Hour Incubation	48 Hour Incubation	24 Hour Incubation	48 Hour Incubation
Terwilliger's Pond	20° C.	45,000,000	65,000,000	37,000,000	51,000,000
Hatchery Bay	"	39,000,000	52,000,000	32,000,000	43,000,000
Lake Erie	"	33,000,000	48,000,000	39,000,000	50,000,000
Put-in-Bay Harbor	"	83,000,000	104,000,000	62,000,000	75,000,000
Portage River	"	40,000,000	59,000,000	33,000,000	44,000,000
Little Portage River	"	84,000,000	110,000,000	60,000,000	76,000,000
Haunck's Pond	"	60,000,000	85,000,000	51,000,000	65,000,000
Terwilliger's Pond	37° C.	54,000,000	74,000,000	45,000,000	60,000,000
Hatchery Bay	"	48,000,000	64,000,000	42,000,000	56,000,000
Lake Erie	"	45,000,000	65,000,000	44,000,000	60,000,000
Put-in-Bay Harbor	"	119,000,000	144,000,000	87,000,000	105,000,000
Portage River	"	53,000,000	73,000,000	46,000,000	61,000,000
Little Portage River	"	115,000,000	150,000,000	80,000,000	99,000,000
Haunck's Pond	"	43,000,000	64,000,000	30,000,000	37,000,000
Terwilliger's Pond	55° C.	39,000,000	50,000,000	26,000,000	38,000,000
Hatchery Bay	"	33,000,000	42,000,000	30,000,000	40,000,000
Lake Erie	"	25,000,000	37,000,000	28,000,000	38,000,000
Put-in-Bay Harbor	"	65,000,000	81,000,000	62,000,000	74,000,000
Portage River	"	48,000,000	61,000,000	46,000,000	60,000,000
Little Portage River	"	97,000,000	122,000,000	75,000,000	97,000,000
Haunck's Pond	"	Not determined	Not determined	Not determined	Not determined

regions. This may mean that the number of organisms necessary to oxidize ammonia to nitrites was too small to bring about the reaction; that the organisms were present but were prevented from acting normally; or that they were absent entirely.

The laboratory data suggested that there were no cellulose-digesting organisms in certain regions, while in other regions certain stations only showed them to be present. (See Table 2). When present, the cellulose was frequently completely digested in about two weeks. The presence of these organisms was noted in 33 stations while they were absent in 19 stations. The cellulose-digesting organisms initiate the destruction of plant materials which can be utilized by living organisms.

The organisms capable of hydrolyzing starch were present in all stations. In 46 stations there was complete hydrolysis while in 9 stations there was partial